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SOME OBSERVATIONS ON THE FORMATION OF GLYCERIC ACID DURING PHOTOSYNTHESIS EXPERIMENTS[1]

D. C. MORTIMER

Abstract

The dephosphorylation of phosphoglyceric acid was tested under a variety of conditions. Hydrolysis of the ester by leaf homogenates was relatively slow, even when conditions favored enzymatic activity, and was not detected when frozen homogenate was extracted by boiling 80% ethanol. The results suggest that glyceric acid recovered in ethanol-soluble extracts of leaves following short-term photosynthesis experiments is not normally derived from phosphoglyceric acid. The route proposed for the synthesis of glyceric acid is related, with particular reference to soybean leaf, to an alternate pathway for photosynthetic assimilation which does not utilize phosphoglyceric acid.

Introduction

Radioactive glyceric acid is one of the products extracted from plants which have photosynthetically assimilated $C^{14}O_2$ for a short time. It is apparently a more prominent product with higher plants than with the algae commonly used in photosynthetic experiments (3). Its presence assumes particular significance in species like the soybean which do not have appreciable amounts of radioactive phosphoglyceric acid among the products of short-term photosynthesis (1, 5). It is generally assumed, although not well documented, that the radioactive glyceric acid recovered from such leaves is an artifact of extraction derived from phosphoglyceric acid.

In most, if not all, experiments of this type, radioactive products are extracted from the plant tissue with hot 80% ethanol or methanol. Although Benson reported (2) that plant phosphatases were inactivated in a few minutes in boiling 80% ethanol, the possibility remains that the activity of the phosphatases was increased sufficiently during the initial contact period to effect the dephosphorylation of phosphoglyceric acid before they were destroyed by the hot ethanol.

Recent iodoacetate inhibition studies (6) showed that partially inhibited sugar beet leaves did not produce glyceric acid although they did accumulate phosphoglyceric acid, while similarly treated soybean leaves continued to pro-

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duce the free acid in the absence of the phosphorylated form. It was important therefore to attempt to establish whether glyceric acid was produced during the extraction or was a normal metabolic product. The results of the experiments reported here favor the latter possibility.

Experimental

The C^{14}-labelled phosphoglyceric acid was isolated from the ethanol-soluble fraction of a sugar beet leaf which had been exposed to $C^{14}O_2$ for 30 seconds. The purification was achieved by band chromatography in different solvents, and radiochemical purity was assessed by two-directional chromatography and autoradiography.

A trifoliate leaf from a 3-week soybean plant (*Glycine max* var. Comet) was frozen in liquid nitrogen and ground to a fine powder. The powder was divided into three parts which were placed in three 50-ml beakers, previously chilled with liquid nitrogen. One hundred microliters of the C^{14}-phosphoglyceric acid (20,000 d.p.m.) was added to each beaker. Fifteen milliliters of boiling 80% ethanol was added to the first beaker, which was immediately placed on the steam bath. Fifteen milliliters of 80% ethanol (25° C) was added to the second beaker, which was then quickly warmed to 25° C, and allowed to stand for 20 minutes before being placed on the steam bath. The frozen mixture of leaf powder and phosphoglyceric acid in the third beaker was warmed to 25° C and allowed to stand for 10 minutes before the boiling 80% ethanol was added and the mixture placed on the steam bath. The three samples were filtered, the filtrates were concentrated, and chromatograms of the extracts were prepared.

A sugar beet leaf which had assimilated $C^{14}O_2$ in the light for 30 seconds was frozen in liquid nitrogen and ground to a powder. The frozen powder was divided between two chilled beakers. Fifty milliliters of boiling, 80% ethanol was added to one and 50 ml of 80% ethanol (25° C) to the other. The "80° beaker" was transferred to the steam bath immediately, while the "25° beaker" was held at 25° C during the 20-minute extraction period. Both samples were filtered, the filtrates were concentrated, and chromatograms of the concentrated extracts were prepared.

Barley leaves (*Hordeum vulgare* var. Brant) were exposed for 60 seconds in the light in an atmosphere containing $C^{14}O_2$, then immersed in liquid nitrogen. In some cases, the leaves were ground, the powder was divided into subportions, thawed, and incubated at room temperature for varying periods before addition of boiling ethanol. In other cases, the leaves were carefully withdrawn from the liquid nitrogen, thawed, incubated in the light at room temperature without prior grinding, then macerated in the Waring Blendor in boiling 80% ethanol.

The methods for preparing the ethanol extract for chromatography, the two-directional chromatography system, and the counting of radioactive areas on the chromatograms have been described (4).

Results

The data in Table I show that radioactive phosphoglyceric acid added to soybean leaf homogenates was not significantly hydrolyzed when the leaf

material was immediately extracted with hot 80% ethanol, or when the extraction was carried out at room temperature. Dephosphorylation was incomplete even if the homogenate was incubated with the radioactive phosphoglyceric acid for 10 minutes at room temperature before the hot ethanol was added.

Sugar beet leaf homogenates tended to be more reactive than the soybean homogenates. If the 80° and 25° C ethanol extraction was used with sugar beet homogenates (Table II), a larger proportion of glyceric acid was recovered in

TABLE I

Effect of extraction procedures on the hydrolysis of phosphoglyceric acid by soybean leaf homogenates

Frozen homogenate plus C^{14}-PGA, extracted in:	% of PGA-C^{14} recovered in glyceric acid
80% ethanol (80° C)	5
80% ethanol (25° C), 20 min, then heated	6
Homogenate thawed, incubated 10 min at 25° C, then 80% ethanol at 80° C	60
Original PGA sample before addition to homogenate	6

TABLE II

Effect of extraction procedure on the hydrolysis of products of 30-second photosynthesis in sugar beet leaf

	Leaf ground in liquid nitrogen, portions extracted in 80% ethanol	
	80° C	25° C
	% of total ethanol-soluble C^{14}	
"Hexose phosphates"	54	38
Phosphoenolpyruvic acid	4	2
Phosphoglyceric acid	26	25
Glyceric acid	1	11
Aspartic acid	2	2
Malic acid	3	3
Serine, glycine	4	5
Sucrose	4	3
Glucose, fructose	0	5
Others	1	5

the 25° extract but the proportion of C^{14} in phosphoglyceric acid was the same in both. The fraction referred to as "hexose phosphates" was presumably the source of the increase in glyceric acid and the small proportion of C^{14} in the free hexoses.

When thawed barley leaves or barley leaf homogenates were incubated for 10 or 30 minutes (Table III), hydrolytic action on the radioactive products of a prior interval of photosynthesis in $C^{14}O_2$ was more pronounced than with sugar beet homogenates. Compared with the control sample, both whole and ground leaves showed reduced proportions of C^{14} in the phosphorylated compounds and sucrose. These were balanced by increases in the C^{14} content of the gly-

TABLE III

Influence of freezing and thawing on the products of 60-second photosynthesis in barley leaves

Ethanol-soluble products	Leaves frozen in liquid nitrogen, then thawed and incubated at 25° C, before and after grinding				
	Control whole or ground leaves, no incubation*	Whole leaves incubated†		Ground leaves incubated†	
		10 min	30 min	10 min	30 min
	% of total ethanol-soluble C^{14}				
"Hexose phosphates"	27	20	19	17	10
Phosphoenolpyruvic acid	3	2	1	1	0
Phosphoglyceric acid	12	6	3	4	0
Glyceric acid	8	28	39	41	48
Serine, glycine	20	17	13	17	17
Glucose-1,2-phosphate	4	1	0	0	0
Sucrose	22	15	0	6	11
Glucose, fructose	0	7	18	8	8
Alanine, threonine	2	4	5	4	6

*Frozen whole or ground leaves were immersed in boiling 80% ethanol.
†The incubation period was the interval between the time that the whole leaves or ground leaves reached 25° C and the time that boiling 80% ethanol was poured over them.

ceric acid and hexose fractions. The ground leaf material hydrolyzed the phosphates more rapidly while the whole leaf inverted or utilized the sucrose more rapidly than the ground. In both samples, however, the proportion of newly formed glyceric acid exceeded, by a considerable amount, the loss of radioactive phosphoglyceric acid.

Discussion

The observation that radioactive phosphoglyceric acid added to soybean leaf homogenates was not hydrolyzed during the normal extraction by boiling 80% ethanol, nor during extraction by cool ethanol and only slowly when incubated with the leaf homogenate before extraction, demonstrated that the glyceric acid recovered from soybean leaves following short-term photosynthesis in $C^{14}O_2$ could not arise by the complete dephosphorylation of phosphoglyceric acid during the extraction by hot 80% ethanol. A similar conclusion may be drawn from the experiments with sugar beet and barley leaves. Under conditions favoring enzymatic degradation of photosynthetic products, additional glyceric acid was formed but the breakdown of phosphoglyceric acid was not great enough to account for the increase.

If phosphoglyceric acid is not the immediate precursor of the free acid isolated from leaves, the actual pathway by which glyceric acid is formed must be established. It also follows that the presence of phosphoglyceric acid in soybean leaves cannot be inferred from the isolation of free glyceric acid. Since phosphoglyceric acid is not normally found in the extracts of soybean leaves following short-term photosynthesis experiments, the carbon dioxide assimilation mechanism in these leaves must occur by a pathway which does not utilize the phosphoglyceric acid reduction step. Analysis of iodoacetate inhibition data (6) has led to the suggestion that glyceric acid is a by-product

resulting from overproduction of the primary fixation and reduction products. The immediate precursor in this scheme is glyceraldehyde-3-phosphate, produced in the sugar beet leaf primarily by the reduction of phosphoglyceric acid, but in the soybean leaf from one of the unstable components of the "hexose phosphate" fraction. The present data are consistent with this scheme.

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MICROBIAL GROWTH FACTORS IN RELATION TO RESISTANCE OF FLAX VARIETIES TO FUSARIUM WILT[1]

A. G. Lochhead and F. D. Cook

Abstract

A comparison was made of growth factor requirements and growth factor synthesizing capacity of bacteria from the root surface and from the seeds of flax varieties resistant and susceptible, respectively, to wilt caused by *Fusarium oxysporum* f. *lini*. The percentage of bacteria from both root and seed which required growth-promoting substances was greater with the resistant ('Bison') than with the susceptible ('Novelty') variety. On the other hand bacteria capable of synthesizing growth factors were proportionately more abundant with the susceptible variety. The findings indicate a more pronounced rhizosphere effect exerted by the susceptible than by the resistant variety, and point to circumstances that favor a greater accumulation (or 'turnover') of vitamins and related substances at the root surface of susceptible plants.

Two strains of *Fusarium oxysporum* f. *lini* capable of inducing wilt required no vitamins for growth; thiamine had a depressing effect. Both strains were able to synthesize various growth factors, though in different degree. Culture filtrates of bacteria from roots of the two varieties of flax showed no difference in their effect on growth of *Fusarium;* however, bacteria from the resistant variety showed somewhat greater ability to depress *Fusarium* than those from the susceptible. Though the findings do not constitute evidence of any relationship between differences in growth factor relationships to pathogenic potential of *Fusarium*, they suggest the value of considering growth-promoting substances in studies of the effect of nutrition on the activity of soil-borne pathogens.

Introduction

Earlier studies in this laboratory, reported in 1940, brought to light certain differences in the 'rhizosphere effect' exerted by varieties of flax resistant and susceptible, respectively, to wilt caused by *Fusarium oxysporum* f. *lini* (10, 17, 23, 28). Not only were the numbers of microorganisms greater in the rhizosphere of susceptible, than of resistant, plants, with corresponding greater 'rhizosphere-soil ratios' (10, 17, 23), but differences of a qualitative nature were noted with respect to the proportions of various morphological and physiological groups of bacteria (10, 17, 28). All comparisons pointed to a more pronounced rhizosphere effect in the case of the susceptible than of the resistant variety (17).

This earlier work embraced similar studies of tobacco varieties resistant and susceptible, respectively, to black root rot caused by *Thielaviopsis basicola*. It was found that the susceptible variety exerted a greater rhizosphere effect, quantitatively and qualitatively, than did the resistant in a manner analogous to that observed with flax (10, 17, 23, 28). Confirmation of the finding that susceptible varieties support larger numbers of microorganisms in the rhizosphere than corresponding resistant varieties has been subsequently reported with other species of crop plants. This was observed by Harper (8) and Rombouts (22) in studies of Panama disease of banana caused by *Fusarium*

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Contribution No. 503, from Microbiology Research Institute, Research Branch, Canada Department of Agriculture, Ottawa, Canada.

oxysporum f. *cubense*, by Buxton (3) with peas varying in resistance to strains of *Fusarium oxysporum* f. *pisi*, and by Agnihothrudu (1) in a comparison of pigeon pea strains susceptible and resistant, respectively, to wilt caused by *Fusarium udum*.

That resistant flax plants, through their root excretions, differ from susceptible plants in their stimulative effects on different groups of fungi was demonstrated in 1941 by Timonin (24). Whereas the susceptible variety selectively favored such genera as *Fusarium* and *Helminthosporium* in the rhizosphere, the resistant variety induced a preferential stimulation of *Trichoderma*, some species of which are able to exert antagonistic effects on various pathogenic fungi. The growth of these same fungal types was affected (in a manner analogous to the influence exerted by root excretions) by hydrocyanic acid, which was found to be liberated in much larger quantities by the resistant variety. The degree of resistance to *Fusarium oxysporum* f. *lini* by flax varieties had been previously reported by Reynolds (20, 21) to be correlated with the amount of hydrocyanic acid recovered from plant tissues.

No information is at hand respecting the growth factor relationships of soil microorganisms in the rhizosphere of resistant and susceptible varieties of plants. However, in recent reports, the rhizosphere effect as such has been investigated. In studies with a number of crop plants (4, 14) it was shown that at or near the roots the proportions of bacteria requiring growth factors were lower than in soil beyond the influence of the plant. On the other hand, the relative incidence of microorganisms capable of synthesizing growth factors was much higher in the rhizosphere than in non-rhizosphere soil (4, 11). In view of the increase in total numbers, bacteria capable of growth factor synthesis may attain very high numbers adjacent to the root.

Since the rhizosphere effect noted appears to favor processes that tend towards a greater accumulation of vitamins at or near the plant root than in soil beyond its influence, the present study was undertaken to note whether resistant and susceptible flax plants varied in respect to the growth factor requirements, as well as the growth factor synthesizing capacity, of the bacteria at the root surface. It is considered that stimulative effects contribute to the changed microbiological equilibrium in the rhizosphere, an equilibrium in which not only saprophytic microorganisms but also microorganisms parasitic to plant roots are necessarily involved.

Material and Methods

In addition to a comparison of bacteria isolated from the root surface (rhizoplane) of uninfected flax plants of the wilt resistant variety 'Bison', and the susceptible variety 'Novelty', the study included a comparison of bacteria isolated from the seed of these varieties. From each of the four sources, approximately 100 cultures were obtained by nonselective plating and isolation procedures previously described (14, 15). Stock cultures were maintained in soil extract yeast semisolid agar.

Growth Factor Requirements

Growth factor requirements of the isolates were determined according to procedures previously outlined (4). In brief, the method involved first the

elimination of all organisms able to grow in a basal medium without growth factors, consisting of, per liter: K_2HPO_4, 1.0 g; KNO_3, 0.5 g; $MgSO_4.7H_2O$, 0.2 g; $CaCl_2$, 0.1 g; NaCl, 0.1 g; $FeCl_3.6H_2O$, 0.01 g; glucose, 1.0 g; and vitamin-free 'Casamino acids', 1.0 g. Organisms unable to develop in the basal medium or in the basal medium supplemented with yeast extract, but able to grow upon the further addition of soil extract, included those requiring vitamin B_{12} or the terregens factor (2, 12, 13, 14, 16), the needs for which were determined by the use of appropriate differential media. All cultures unable to grow in the basal medium were tested for specific vitamin requirements (other than for B_{12} or TF) by use of a series of media containing suitable combinations of vitamins to note the effect of the omission of each vitamin in turn. These included thiamine, biotin, pantothenic acid, folic acid, nicotinic acid, riboflavin, pyridoxine (with pyridoxal and pyridoxamine), *p*-aminobenzoic acid, choline, and inositol at concentrations used previously (4). Suitable negative and posi-

TABLE I

Indicator organisms and media for detection of growth factors in culture filtrates

Culture No.	Growth factor tested for	Negative control medium (in addition to salts and glucose)	Positive control medium (addenda to neg. control)
824 (*Arthrobacter* sp.)	Thiamine	'Casamino acids'	Thiamine (50 μg/100 ml)
54 (*Micrococcus* sp.)	Biotin	'Casamino acids' thiamine vitamin B_{12}	Biotin (0.1 μg/100 ml)
S.101 (*Flavobacterium* sp.)	Pantothenic acid	'Casamino acids' thiamine biotin	Ca pantothenate (50 μg/100 ml)
GR.28 (*Agrobacterium* sp.)	Folic acid	'Casamino acids' thiamine biotin Ca pantothenate nicotinic acid	Folic acid (10 μg/100 ml)
Ci 3 (*Corynebacterium insidiosum*)	Nicotinic acid	'Casamino acids' thiamine biotin Ca pantothenate folic acid	Nicotinic acid (50 μg/100 ml)
785 (*Flavobacterium* sp.)	Riboflavin	'Casamino acids'	Riboflavin (50 μg/100 ml)
GR.94 (*Arthrobacter* sp.?)	Pyridoxine	'Casamino acids' thiamine biotin Ca pantothenate folic acid nicotinic acid	Pyridoxine (50 μg/100 ml) Pyridoxal (50 μg/100 ml) Pyridoxamine (10 μg/100 ml)
541 (*Arthrobacter duodecadis*)	Vitamin B_{12}	Yeast extract	Vitamin B_{12} (0.2 μg/100 ml)
401 (*Arthrobacter flavescens*)	Terregens factor	Yeast extract	Terregens factor (10 μg/100 ml)

tive controls were included. A growth factor was considered essential if no growth occurred on its omission from an otherwise suitable medium. In doubtful cases serial transfers were made to eliminate any possible carry-over effect.

Synthesis of Growth Factors

The ability of each isolate to synthesize one or more of nine growth factors was determined by procedures described in more detail in previous publications (4, 11). The growth factors consisted of thiamine, biotin, pantothenic acid, folic acid, nicotinic acid, riboflavin, pyridoxine, vitamin B_{12}, and the terregens factor. The culture medium was similar to the basal medium described above with the addition, per liter, of 8 mg $CoCl_2.6H_2O$ and 4.0 g, instead of 1.0 g, of 'Casamino acids'. Following incubation of the cultures (40-ml quantities in 125-ml Erlenmeyer flasks) on a shaker for 6 days at 26° C, the culture fluids, after being clarified and sterilized, were examined for the presence of each of the growth-promoting substances.

The general procedure for recognition of the growth factors consisted in adding to 5.0 ml of a suitable basal medium (negative control), adequate for growth of the test organism except for the factor in question, 0.5 ml of the clear metabolic liquid and inoculating the medium with the appropriate indicator organism. The latter were chosen on the basis of their specific nutrilite requirements. Definite growth of the indicator organism in the presence of the filtrate was considered to indicate the presence of the factor in question. Negative and positive controls, the latter containing the factor under test, were carried in all cases, the composition of which varied according to the requirements of the test organism (Table I).

Results

Growth Factor Requirements

A summary of the growth factor requirements of the bacteria isolated from the seed and the root surface of the two varieties of flax is presented in Table II. With organisms from both sources, seed and root, the percentage of those requiring one or more growth factors was greater with the resistant than with the susceptible variety of flax. As previously indicated, previous studies (4, 14) had shown that in the rhizosphere the proportion of bacteria requiring growth factors is lower than in non-rhizosphere soil. Thus the rhizosphere effect is more pronounced in the case of the susceptible variety, a finding that supports results of early work in which other criteria were used to compare susceptible and resistant varieties of plants with respect to their relationship to the soil microflora.

Of the 388 isolates examined eight cultures were found to require a factor or factors present in yeast extract the nature of which has not been determined. Regarding more specific growth factor requirements, thiamine and biotin were the vitamins most frequently required by the organisms from the root surface. However, with the bacteria characteristic of seed, pantothenic acid was found to be by far the most frequently needed vitamin. The hypothesis that pantothenic acid is specially important in the nutrition of microorganisms associated with seed is supported by previous, unreported findings of studies

TABLE II

Growth factor requirements of bacteria from seed and roots respectively of 'Bison' (resistant) and 'Novelty' (susceptible) varieties of flax

Growth factor required	'Bison' seed (103 isolates)				'Novelty' seed (88 isolates)				'Bison' roots (92 isolates)				'Novelty' roots (105 isolates)			
	Alone	With others	Total	%	Alone	With others	Total	%	Alone	With others	Total	%	Alone	With others	Total	%
Thiamine	0	4	4	3.9	0	2	2	2.3	8	15	23	25.0	4	7	11	10.5
Biotin	0	1	1	1.0	0	0	0	1.1	4	11	15	16.3	2	3	5	4.8
Pantothenic acid	17	6	23	22.3	7	2	9	10.3	2	5	7	7.6	0	2	2	1.9
Folic acid	0	2	2	1.9	0	0	0	<1.1	0	6	6	6.4	0	2	2	1.9
Nicotinic acid	0	0	0	<1.0	0	1	1	1.1	0	1	1	1.1	0	0	0	<1.0
Riboflavin	0	0	0	<1.0	0	0	0	<1.1	0	0	0	<1.1	1	0	1	1.0
Pyridoxine	0	0	0	<1.0	0	0	0	<1.1	0	0	0	<1.1	0	0	0	<1.0
Vitamin B_{12}	0	0	0	<1.0	0	0	0	<1.1	0	3	3	3.3	0	2	2	1.9
Terregens factor	0	0	0	<1.0	0	0	0	<1.1	0	0	0	<1.1	0	0	0	<1.0
One or more			23	22.3			9	10.3			31	33.7			14	13.3
Undetermined factor in yeast extract			1				5				2				0	

with bacteria from wheat. Whereas this vitamin has never been found as the most frequently required growth factor for bacteria from soil, rhizosphere, or rhizoplane, it is of special significance for seed organisms, as illustrated in Table III. In this connection the results of Louis (18) are of interest; in a study of pantothenic acid in the pea plant he found that this vitamin, which occurs in all sections during the earlier stages of growth, decreases with the age of the plant in the roots and aboveground parts and at the time of ripening is found only in the seed.

TABLE III

Frequency of requirements for pantothenic acid, thiamine, and biotin by bacteria isolated from seed and other sources

Sample	No. of isolates tested	Ranking order of requirement (nine growth factors)		
		Pantothenic acid	Thiamine	Biotin
Seed				
Wheat (series 1, 1957)	108	1	3	2
Wheat (series 2, 1957)	110	1	4	2
Flax ('Bison', 1959)	103	1	2	4
Flax ('Novelty', 1959)	88	1	2	4
Non-rhizosphere soil				
Field soil (1955)	499	4	1	2
Greenhouse soil (1957)	74	3	1	2
Field soil (1958)	107	5	1	3
Rhizosphere				
Wheat (series 1, 1957)	100	4	2	1
Wheat (series 2, 1957)	90	6	2	1
Wheat (series 3, 1957)	103	3	1	2
Wheat (1958)	99	4	1	2
Rhizoplane				
Wheat (series 1, 1957)	100	4	1	3
Wheat (1958)	100	7	1	2
Flax ('Bison', 1959)	92	3	1	2
Flax ('Novelty', 1959)	107	3	1	2

Synthesis of Growth Factors

The capabilities of the organisms isolated from the seed and the roots, respectively, of the two flax varieties for synthesis of nine growth factors are shown in Table IV. Although in all cases the majority of the isolates were able to produce one or more growth factors, this ability was more pronounced with the 'Novelty' organisms, from both seed and root. The greater capacity for synthesis was seen not only in the proportions able to produce one or more factors, but also in the ability of the 'Novelty' isolates to synthesize a greater average number of growth factors than those of the 'Bison' variety. Since it has been shown (4, 11) that increased capacity for growth factor synthesis by microorganisms is one feature of the rhizosphere effect, the data in Table IV with respect to the root isolates give further confirmation of a greater rhizosphere effect exerted by the susceptible than by the resistant variety.

Respecting specific vitamins, the seed organisms were characterized by an unusually low capacity for the synthesis of vitamin B_{12}, not apparent with the

TABLE IV

Growth factors produced by bacteria from seed and roots respectively of 'Bison' (resistant) and 'Novelty' (susceptible) varieties of flax

Growth factor produced	'Bison' seed (103)		'Novelty' seed (88)		'Bison' roots (92)		'Novelty' roots (105)	
	No.	%	No.	%	No.	%	No.	%
Thiamine	60	58.3	71	80.7	57	62.0	74	70.5
Biotin	73	70.9	71	80.7	12	13.0	63	60.0
Pantothenic acid	64	62.1	68	77.3	61	66.3	87	82.9
Folic acid	66	64.1	66	75.0	46	50.0	79	75.2
Nicotinic acid	86	83.5	76	86.4	54	58.7	65	61.9
Riboflavin	66	64.1	71	80.7	50	54.3	94	89.5
Pyridoxine	45	43.7	46	52.3	15	16.3	46	43.8
Vitamin B_{12}	5	4.8	2	2.3	23	25.0	43	41.0
Terregens factor	18	17.4	10	11.4	28	30.4	30	28.6
One or more	87	84.4	79	89.8	72	78.3	96	91.4
1 factor	3	2.9	2	2.3	0	0.0	0	0.0
2 factors	3	2.9	1	1.1	8	8.7	2	1.9
3 factors	0	0.0	1	1.1	11	12.0	5	4.7
4 factors	10	9.7	2	2.3	12	13.0	7	6.5
5 factors	18	17.5	14	15.9	13	14.1	14	13.1
6 factors	35	34.0	22	25.0	16	17.4	27	25.2
7 factors	13	12.6	32	36.4	7	7.6	26	24.3
8 factors	4	3.9	5	5.7	5	5.4	15	14.0
9 factors	1	1.0	0	0.0	0	0.0	0	0.0
Average number of growth factors synthesized per active culture	5.5		6.1		4.8		6.0	

root isolates. Although the 'Novelty' root bacteria showed higher percentages capable of producing every growth factor except TF, the differences were most pronounced with biotin and pyridoxine.

Observations

Although the findings suggest that the flax varieties resistant and susceptible, respectively, to *Fusarium* wilt differ in the degree of their rhizosphere effect in respect to the growth factor requirements and synthetic ability of the bacteria associated with their root surface, yet the data constitute no evidence in support of any causal relationship of the phenomena observed to disease resistance. Any demonstration of this must await further experimentation.

The lower proportion of growth factor requiring bacteria associated with the susceptible variety, considered in conjunction with the higher capacity for synthesis of growth-promoting substances, points to circumstances that favor a greater accumulation of such factors at the root surface of the susceptible than of the resistant variety. While it is interesting to speculate that differences in available vitamins might affect the pathogen by modifying its capacity for growth or pathogenicity, it should be kept in mind that, in the rhizosphere, direct relationships cannot as yet be well evaluated, for any substance produced by a given segment of the microflora which may stimulate or depress a pathogenic form may equally well stimulate or depress other members of the

soil microflora, which may in turn influence the pathogen. Thus it is possible that an indirect effect of a given factor could be opposite to its effect if exerted directly, e.g. under circumstances free from the associative and antagonistic influences to which the many groups of microorganisms are exposed in natural soil and which are accentuated in the rhizosphere. However, the results suggested the value of some exploratory tests on the growth factor relationships of *Fusarium oxysporum* f. *lini* as well as certain aspects of mutual influences of this organism and the root bacteria.

Tests with *Fusarium oxysporum* f. *lini*

General Procedure

Two strains of *Fusarium* were used, M-18 and M-32, isolated respectively in Quebec and Saskatchewan and both capable of causing wilt in flax. The basal medium contained, per liter: sucrose, 25.0 g; KH_2PO_4, 1.0 g; $MgSO_4.7H_2O$, 0.5 g; KCl, 0.05 g; $CaCl_2$, 0.02 g; $FeCl_3.6H_2O$, 5.0 mg; and a mixture of salts of trace elements containing Zn, Cu, Mn, Mo, and B. Two modifications, depending on the nitrogen source, were used containing, respectively, $NaNO_3$, 1.5 g (medium A) and vitamin-free 'Casamino acids', 2.0 g per liter (medium B). For studies of vitamin synthesis $CoCl_2$, 8.0 mg per liter, was used.

In tests of vitamin effects and associative action on the growth of *Fusarium* the media were dispensed in 25-ml amounts in 125-ml flasks inoculated with 1.0 ml of a spore suspension in distilled water, prepared from agar slants of medium A. Following stationary incubation at 26° C the cultures were filtered through a 200-mesh copper wire screen and the mycelial mats placed in tared aluminum dishes, dried overnight at 90° C, and weighed.

Similar procedures were used for studying the effect of culture filtrates of bacteria from the root surface, as well as the effect of associated growth of the bacteria, on the development of *Fusarium*. In the filtrate tests 1.0 ml of sterile metabolic culture fluid (as described above for growth factor synthesis by bacteria) was added to each flask prior to inoculation with the fungus. In the series on associated growth, simultaneous inoculation was made of *Fusarium* and 1.0 ml of culture of the bacterial isolate being tested. Suitable additions were made to all control flasks to compensate for the ingredients used in the filtrates or the bacterial inocula.

For testing growth factor synthesis, 80-ml amounts of medium were used in 300-ml flasks. Following inoculation and stationary incubation, the cultures were heated, clarified by centrifugation, and sterilized. The presence of growth factors in the clear metabolic fluids was determined by the method described above for bacterial cultures, in which the same indicator organisms were used.

Effect of Vitamins on Growth of Fusarium

The effect of eight vitamins, at concentrations similar to those indicated in Table I, on the growth of *F. oxysporum* f. *lini* is shown in Table V. For neither strain of the organism were vitamins found to be essential or stimulatory. Little or no effect on growth was noted except in the presence of thiamine, which exerted a depressing action.

With a number of fungal types, including species of *Fusarium*, inhibitory effects of thiamine on growth have been reported, as in the case of *Fusarium*

TABLE V

Effect of vitamins on growth of *Fusarium oxysporum* f. *lini*

Addenda to basal medium	Mat weight (mg), average of three flasks 12 days, 26° C			
	Basal medium—salts, sucrose, NO_3-N		Basal medium—salts, sucrose, amino-N	
	Strain M-18	Strain M-32	Strain M-18	Strain M-32
Expt. 1				
No vitamins	129	115	139	98
Eight vitamins	119	101	123	75
Eight vitamins minus biotin	114	96	120	74
Biotin	132	110	134	96
Expt. 2				
No vitamins			154	84
Eight vitamins			94	66
Thiamine			99	75
Biotin			136	84
Pantothenic acid			139	85
Folic acid			139	84
Nicotinic acid			140	83
Riboflavin			131	83
Pyridoxine			143	85
Inositol			142	83

lini (29), *Fusarium avenaceum* (5), and *Fusarium solani* (26). With *F. solani* the depressing effect of thiamine was found by Turel (26) to depend upon the culture medium. In Richard's medium (glucose and inorganic salts) thiamine, at concentrations between 10 μg and 1000 μg per liter, caused a depression following an initial stimulation of growth. On the other hand no inhibitory effect was observed in Raulin's solution.

Synthesis of Growth Factors by Fusarium

The ability of the two strains of *Fusarium oxysporum* f. *lini* to synthesize nine growth factors in two culture media is shown in Table VI. As expected,

TABLE VI

Synthesis of growth factors by two strains of *Fusarium oxysporum* f. *lini* in media with inorganic and amino nitrogen respectively

Growth factor	Strain M-18				Strain M-32			
	Medium A (inorganic N)		Medium B (amino N)		Medium A (inorganic N)		Medium B (amino N)	
	7 days	14 days	7 days	14 days	7 days	14 days	7 days	14 days
Thiamine	−	−	+	−	++++	+++	+++	++++
Biotin	−	−	++	+	++++	++++	++++	++++
Pantothenic acid	+	++	+++	+++	+++	++++	++++	+++
Folic acid	−	−	++++	++++	++++	++++	++++	++++
Nicotinic acid	−	++	++++	++++	++++	++++	+++	+++
Riboflavin	++	++	+++	+++	++++	++++	+++	++++
Pyridoxine	−	−	−	−	++++	−	−	−
Vitamin B_{12}	−	−	−	−	−	−	−	−
Terregens factor	−	−	+	++	++++	+++	+++	++++

NOTE: + signs indicate relative amounts (positive control = ++++); − sign indicates no growth factor detected.

neither of the strains was able to produce vitamin B_{12}. Although the detection of growth factors depended upon the length of incubation and varied with the medium employed, the findings indicate that different strains of the *Fusarium* may vary with respect to their ability for vitamin synthesis and that some strains such as M-32 may have a pronounced capacity for elaborating growth-promoting substances.

Associative Effects—Root Surface Bacteria and Fusarium

The effects of the culture filtrates, as well as the associated growth, of the bacteria isolated from the root surface of the resistant and susceptible flax varieties on the growth of *F. oxysporum* f. *lini* (M-18) are summarized in Table VII. The findings are expressed in terms of increases or decreases in mat weights compared with those of the controls. No difference between the flax varieties was noted with respect to the effect of the culture filtrates. With the tests on associated growth a somewhat higher proportion of the isolates from the resistant variety was able to exert a depressing action on the growth of *Fusarium*.

TABLE VII

Effect of culture filtrates and of associated growth of bacteria from root surface of resistant and susceptible flax varieties on growth of *Fusarium oxysporum* f. *lini* (M-18)

	Bacteria from 'Bison' (R)		Bacteria from 'Novelty' (S)	
	No.	%	No.	%
Effect of sterile culture filtrates				
Total number of filtrates tested	84		97	
Depressing effect on *Fusarium*	23	27.4	27	27.8
No effect	2	2.4	2	2.1
Stimulative effect	59	70.2	68	70.1
Effect of associated growth				
Total number of isolates tested	86		101	
Depressing effect on *Fusarium*	49	57.0	50	49.5
No effect	1	1.2	1	1.0
Stimulative effect	36	41.8	50	49.5

Discussion

In any consideration of the relationship of growth factors to the pathogen it cannot be too strongly emphasized that growth is not synonymous with pathogenicity. However, the apparent potentiality for accumulation of higher concentrations, or greater 'turnover', of vitamins at or near the root surface of the susceptible, than the resistant, variety suggests the possible value of examining any relationship between this phenomenon and the capability of the pathogen for inducing disease, a subject outside the scope of this report.

That the pathogenic potential of certain fungi may vary with the nutritional environment and change according to the substrates on which they are cultivated prior to tests for pathogenicity is a concept that has been supported by various studies (cf. Gäumann (7), Zogg (31)). Hrushovetz (9) noted that

attenuation of the virulence of *Helminthosporium sativum* to wheat seedlings occurred after successive transfers to media containing various amino acids; the effect was particularly marked with *dl*-methionine. This amino acid had been previously noted by Wallace and Lochhead (27) to be by far the most essential compound for amino acid-requiring bacteria in the rhizosphere. In the rhizosphere of the susceptible variety, which normally supports a greater microbial population than does the resistant variety, less methionine might well be available for the *Fusarium* through its increased utilization by competing bacteria. Competition for nutrients has been suggested by Marshall and Alexander (19) as being one of the major factors governing the biological control of soil-borne fungi, as a result of their studies of interactions between *Fusarium oxysporum* f. *cubense* and various soil bacteria.

That the nutrition of the pathogen may play a significant role in the manifestation of pathogenesis has been recently emphasized by Toussoun *et al.* (25). Studies with the soil-borne pathogen *Fusarium solani* f. *phaseoli* showed that the establishment of pathogenic relationship was enhanced by nitrogen, organic sources being more effective than inorganic sources; on the other hand this relationship was delayed by glucose.

Respecting the specific effects of vitamins or other growth factors in inducing changes in the virulence of pathogenic fungi, fewer reports appear to be available. Flück (6) reported an increase in virulence of *Ophiobolus graminis* after growing the organism in a medium with thiamine; biotin was without effect. In a series of studies of the effect of graded concentrations of thiamine and biotin on the growth of *Calonectria graminicola* in synthetic liquid medium and on its pathogenicity for wheat plants following infection with the cultures, Zogg (30, 32, 33) reported effects attributable to the vitamins. Biotin caused a depression in the disease index, particularly at concentrations of 5 to 50 μg/l.; on the other hand thiamine had the effect of increasing the disease index. In an extension of the studies (33) it was noted that the effect of biotin was dependent on the temperature of incubation and on the biotin concentration. Whereas at concentrations up to 5μg/l. the pathogenic potential rose, with increasing temperature an inversion occurred between 5μg and 50μg/l. so that above 50μg/l. there was a decline in pathogenicity with rising temperature. In no case was a relationship observed between the pathogenicity and the saprophytic growth of the organism.

Though the present study is concerned chiefly with differences in 'growth factor potential' at or near the root surface of varieties of plants varying in disease resistance related to the characteristics of the saprophytic microflora, the findings, in the light of work referred to, suggest the value of giving further consideration to vitamins or other growth accessory substances in studies of the effect of nutrition on the activity of soil-borne pathogens.

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CYTOTAXONOMY, PHYLOGENY, AND CANADIAN DISTRIBUTION OF CIRSIUM UNDULATUM AND CIRSIUM FLODMANII[1]

C. Frankton and R. J. Moore

Abstract

The morphology and specific differences of *Cirsium undulatum* (Nutt.) Spreng. and of *C. flodmanii* (Rydb.) Arthur are described and their Canadian distributions are reported in detail. The chromosome numbers are *C. undulatum* f. *undulatum* and f. *album* Farwell, $2n=26$; *C. flodmanii* f. *flodmanii* and f. *albiflorum* D. Löve, $2n=22$. The origin of four North American species of *Cirsium* that do not follow the world-wide base number 17 is discussed; it is postulated that reduction in number has occurred by translocations. The chromosomes of species with reduced numbers are larger than those of the unreduced species but the total length of the chromosomes of both groups is approximately the same.

Introduction

Some 10 years ago a general study of the Canadian species of *Cirsium* was begun. At that time the genus was poorly represented in the herbarium of the Department of Agriculture and also in other Canadian herbaria visited. With the co-operation of field parties, collections have now reached a point where it is possible to discuss the distribution and cytotaxonomy of some of the species found in Canada. This first paper will deal with two species native to the prairies of western North America: *Cirsium undulatum* (Nutt.) Spreng. and *C. flodmanii* (Rydb.) Arthur. These species have been so misunderstood, particularly in Canadian floras, that a rather full discussion of morphology is necessary in order to establish exactly what plants are being dealt with and to draw attention to the types of characters that may be useful in the study of *Cirsium*.

Morphology of the Species

Specimens of *Cirsium undulatum* and *C. flodmanii* are very often misidentified in herbaria and most frequently are found filed as *C. discolor* (Muhl.) Spreng., *C. altissimum* (L.) Spreng., and *C. vulgare* (Savi) Tenore. Both *C. undulatum* and *C. flodmanii* are woolly-stemmed, alone a character sufficient for differentiation from *C. discolor* and *C. altissimum*. The introduced thistle, *C. vulgare*, is the only Canadian thistle with spines on the leaf surface and therefore should not present any difficulty in identification. The combination of densely tomentose stems, leaves densely tomentose at least on the lower surface, flowers purple, only rarely white, and glandular sticky involucral bracts separate both *C. undulatum* and *C. flodmanii* from all other thistles of Canada. In addition to these characters, the inner involucral bracts are not dilated at their apices and the heads are not cobwebby-pubescent. Ownbey (1952) gives a very detailed description of *C. undulatum* together with a photograph of the type.

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The following comparison of *C. undulatum* and *C. flodmanii* will bring out characters reliable in their separation.

Authors usually differentiate these species on involucral size and leaf characters. The cauline leaves of *C. flodmanii* are described as being more deeply pinnatifid with lobes less than 7 mm broad whereas the lobes of the leaves of *C. undulatum* are broader. Certainly leaf criteria are sufficiently reliable to separate most specimens but extremes are found that are difficult to separate on leaf characters alone. Specimens of either species may have subentire leaves, and basal leaves of *C. flodmanii* with lobes broader than 7 mm are not infrequent.

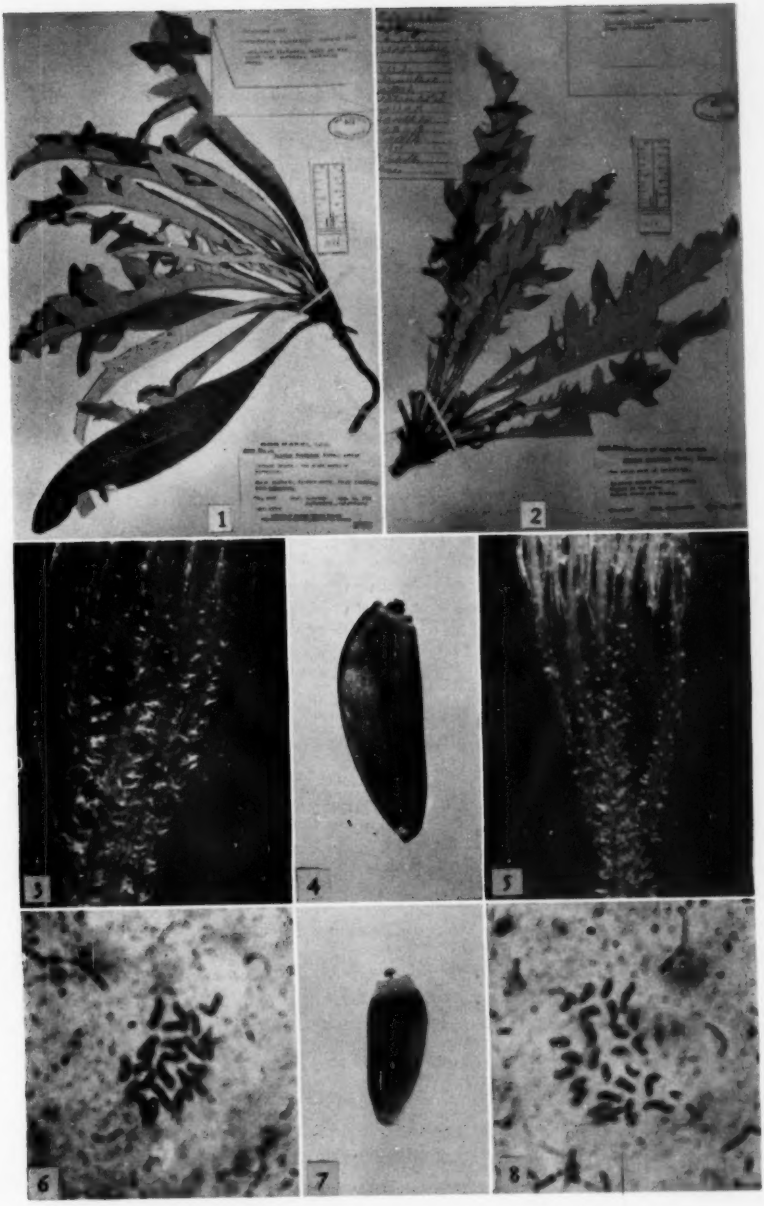
It is in the rosette that the best foliage differences are found. Collections of *Cirsium* rarely include rosettes, but the importance of the rosette in the separation of *Cirsium* species should be emphasized. In the present case, rosette characters are so diagnostic that the species can be separated in the field or in the herbarium with great ease. The rosette leaves of *C. flodmanii* are greenish above, frequently entire or remotely lobed (Fig. 1). The first leaves of *undulatum* rosettes may be almost entire but generally the leaves are strongly lobed, the lobes overlapping and directed at an upward angle from the surface of the leaf (Fig. 2). The leaves are always densely tomentose on both surfaces. Both species have the capacity to develop offshoots but this tendency is greater in *C. flodmanii* and the resulting larger patches of greener rosettes can be readily distinguished from those of *C. undulatum* even from a rapidly moving vehicle.

The involucre of *C. undulatum* is, in general, larger, but not invariably so, and reliance on involucral size has led to misidentification, as Ownbey (1952) has pointed out. The involucre of this species is coarser, rather broader at the base and in dried specimens usually appears pressed less flat than that of *C. flodmanii*. The bracts of the latter species are usually more closely imbricated and, as a result, the heads in dry specimens appear darker because of the closer juxtaposition of the glandular ridges. Flowers of *C. flodmanii* are a deeper brighter purple, a difference particularly marked in herbarium material. *Cirsium undulatum* usually begins to flower before *C. flodmanii* and seeds of the former are mature from July 15 on, whereas seed of *C. flodmanii* is rarely ripe before August.

Achene characters are particularly valuable for the separation of these species. The achenes of *C. flodmanii* are 3.5–5 mm (average 4 mm) long, excluding the callus, and 1.5–2 mm broad (Fig. 7). They are usually yellowish-brown in color and have an apical yellow or yellow-white band extending to a depth of ½ mm. The achenes of *C. undulatum* (Fig. 4) are brown and measure 5–7 mm (average 6 mm) in length and 2–3 mm in width. The light-

FIG. 1. Rosette of *C. flodmanii.*
FIG. 2. Rosette of *C. undulatum.*
FIG. 3. Filament hairs of *C. flodmanii.*
FIG. 4. Achene of *C. undulatum,* ×6.
FIG. 5. Filament hairs of *C. undulatum.*
FIG. 6. Somatic chromosomes of *C. flodmanii,* ×4800.
FIG. 7. Achene of *C. flodmanii,* ×6.
FIG. 8. Somatic chromosomes of *C. undulatum,* ×4800.

PLATE I



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colored apical band is lacking or is very narrow and only very rarely reaches $\frac{1}{3}$ mm in depth.

It might be expected that characters of individual florets would be of value in the differentiation of these species. The florets and the anthers, including the caudal appendage, of *C. undulatum* tend to be longer than those of its congener. However, the size ranges found in the two species overlap to such an extent that these characters are of little value, except in the case of specimens at the extremes of the range. The range of measurements is shown in Table I. Specific differences in filament hairiness are obvious in most specimens. When the corolla is opened, the filaments in the majority of specimens of *C. flodmanii* appear to be densely matted with long hairs whereas the filament hairs of *C. undulatum* are short and usually sparse (Figs. 3, 5). Experience is required to interpret this character but a random test indicates that it may be extremely useful in dividing the species.

TABLE I

Size of florets and anthers

Species	No. of plants	Length of anther (mm)			Length of florets (mm)		
		Shortest	Longest	Average	Shortest	Longest	Average
flodmanii	72	6.5	11.8	9.3	21.1	36.2	28.4
undulatum	39	9.4	13.3	11.2	27.0	40.0	31.9

An example of the use of some of these characters in the separation of two superficially similar specimens will serve to summarize the important differences. A specimen of *C. flodmanii* from just north of Riceville, Montana (*Senn, Frankton and Gillett 5657*), and one of *C. undulatum* from Lethbridge, Alberta (*Frankton 1342*), were collected within 3 days of each other in 1951. Both have nearly entire cauline leaves with broad based shallow lobes. Although ordinarily *C. undulatum* flowers and produces seed earlier than its congener, in this case both collections are at the same stage of development. Heads and involucral bracts in both are almost identical in size; the *flodmanii* specimen shows the usual greater widening of the involucre from base to apex. The criteria generally employed are of little use in separating these specimens, but here the rosettes are of great value and, as can be seen in Figs. 1, 2, clearly reveal the specific differences. The seeds of the *undulatum* specimen are 6 mm long, over 2 mm broad, and lack an apical light-colored band; those of the *C. flodmanii* measure 4.5 mm long and less than 2 mm broad and have a prominent apical yellow band. The usual mass of long hairs is found on the stamen filaments of *C. flodmanii*; only short sparse hairs occur on those of the other.

Distribution of the Species

The Canadian distributions of *C. undulatum* and of *C. flodmanii* are shown in Figs. 9, 10. These maps were compiled from specimens on file in the herbarium of the Department of Agriculture, Ottawa (DAO) and in the National

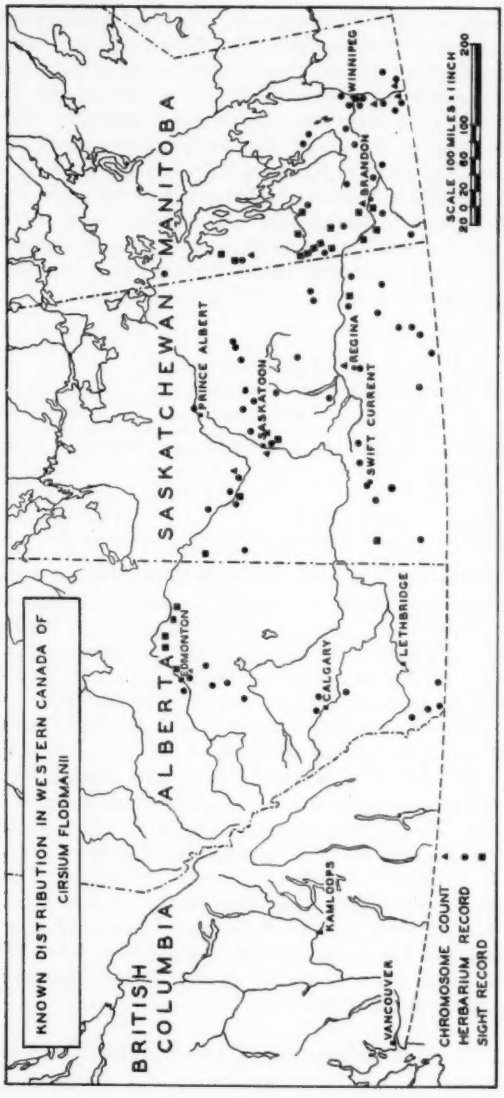

FIG. 9. Distribution of *C. flodmanii* in Canada.

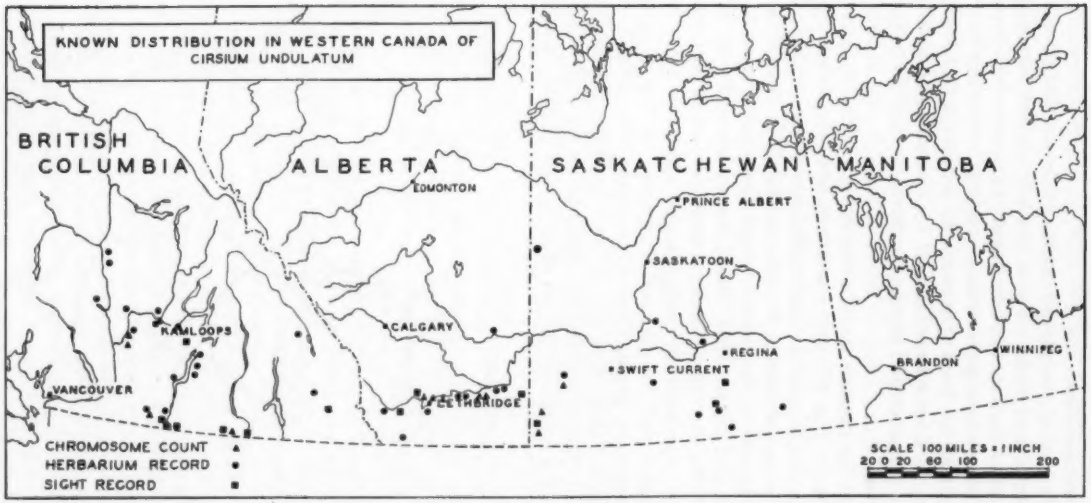

FIG. 10. Distribution of *C. undulatum* in Canada.

Herbarium, Ottawa (CAN). As indicated by the symbols, sight records are also included. The sources of the plants for which chromosome counts are here reported are indicated.

It will be seen that *C. undulatum* occurs in the southern portions of British Columbia, Alberta, and Saskatchewan but has not been found in Manitoba. *Cirsium flodmanii* does not occur in British Columbia but has a much wider distribution in the prairie provinces, extending farther north and east, than *C. undulatum*. Although both species occur in the Central Plains of Canada, they rarely are found in close proximity. *Cirsium undulatum* is found on drier sites than is its congener and in the northerly part of its range is limited to dry coulee banks.

In addition to the western distribution shown in Fig. 9, *C. flodmanii* occurs in Eastern Canada (Fig. 11) and specimens from the following locations are at hand (DAO, CAN): ONTARIO—Malachi, Port Arthur, Fort William, Port Sydney, Temagami Forest Reserve; QUEBEC—Nomininge, St. Jovite. Fernald (1943) reported the occurrence of this species near Minerva, Essex Co., New York, and Pease (1944) found it near Island Pond, Brighton, Essex Co., Vermont. Whether these eastern sites are native stations or represent recent migration eastward through human agency is not clear. Raymond (1950, p. 48) has noted the unusual occurrence at Nomininge, Quebec, of several western species, among them *C. canescens*, now revised to *C. flodmanii*. Raymond suggested that these plants were probably introduced by Indians. The Ontario and Quebec specimens extend in an arc that suggests that they may be relicts of a more continuous distribution of early postglacial time.

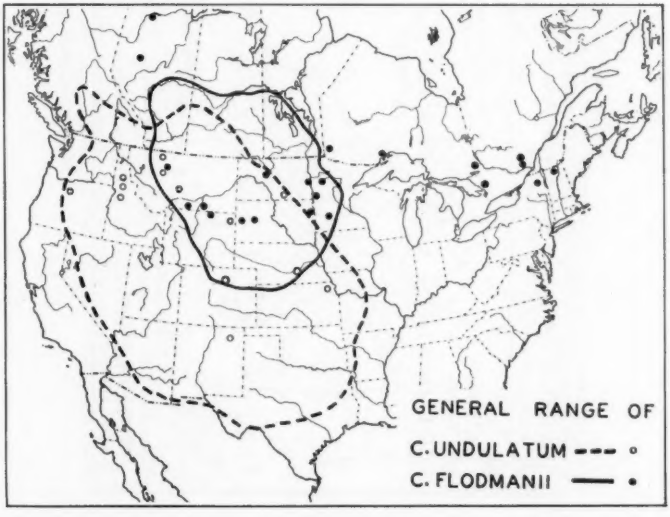

FIG. 11. General distribution of *C. flodmanii* and *C. undulatum*.

The white-flowered form of *C. flodmanii* was reported by its author, D. Löve (1953), from Langruth and Letellier, Man.; Tyvan, Sask., and from 10 mi SE. of Browning, Mont., U.S.A. We have additional records (DAO) from Gladstone, Man.; Camrose, Alta.; Rapid City and Spearfish, S.D. (latter as *C. foliosum* Hook., Bennett 4738) and New Ulm, Minn. Löve is supported in her contention that this form is rare but with a distribution similar to that of the species. We have seen fewer collections of white-flowered *undulatum* but have records from three sites: 10 mi E. of Maple Creek, Sask., SE. of Newcastle, S.D.; 10 mi N. of White Sulphur Springs, Mont.

The general distribution of the two species is shown in Fig. 11. The range in the United States was generalized from material available at Ottawa and from ranges given in floras of the various states or regions. The locations of available collections from the United States is indicated by symbols. It will be noted that *C. flodmanii* has extended only as far westwards as the Continental Divide. However, *C. undulatum* is found both east and west of the Divide but apparently has not crossed the Cascade Range to reach the Pacific Coast.

Cytology

Materials and Methods

Seed and pressed specimens have been obtained through the co-operation of many persons, chiefly members of our staff who were travelling or making botanical surveys in western Canada. In some cases seed on herbarium specimens up to 6 years old has been found to be viable. The seedlings have been grown in greenhouse and field plots at Ottawa.

Specimens are preserved in the herbarium of the Department of Agriculture, Ottawa (DAO). These include the original field collections from which seed was taken and sometimes, as well, the seedling on which the chromosome count was made. In some cases the seedling died from disease before flowering.

Root tips from potted plants were placed in vials of tap water in a refrigerator at 2–3° C for approximately 2 hours, a treatment which shortens the chromosomes very slightly and renders morphology more distinct. The roots were fixed in Randolph's Navashin fixative, embedded in paraffin, sectioned, and stained in crystal violet in the usual manner.

Chromosome morphology was studied with regard to the length of the chromosome only. It was not always possible to determine with certainty the position of the centromere in such small chromosomes and it was felt that to attempt to do so would probably introduce considerable error. Metaphase plates in which the chromosomes lay flat with a minimum of overlapping were selected and drawn by camera lucida at a magnification of 3500.

The drawings were measured with a compass, use of which made it possible to measure accurately each segment of a chromosome which did not lie in a straight line. Measurements were made to .5 mm and were converted into microns to give the actual size of the chromosomes. The latter figure was taken to one decimal point. Experimental error in making and measuring a chromosome drawing probably lies within .5 mm, an amount equivalent to

.14 μ of actual size and it is therefore judged that the measurements may be regarded as accurate only within .2 μ.

From two to five metaphase plates were studied for each plant. The lengths of the chromosomes of each of the cells of one plant were arranged in series of increasing magnitude and an average length, to the nearest .5 μ, was determined for chromosomes of corresponding size rank. The averages for plants of the same species were compared and a species average was calculated for each chromosome. The total length of all chromosomes per cell was determined and plant and species averages were calculated.

It is realized that chromosome size variation may be due to such environmental factors as the growing condition of the plant, the exact stage of mitosis (early or late metaphase) in the cells studied, and variations in the air temperature and in the temperature and duration of the prefixation cold treatment. Comparisons of the various measurements were therefore made to decide whether these results were sufficiently consistent to warrant dependence on them as demonstrating a valid genetic feature. Reliability of the measurements was evaluated by consideration of the variation which existed at each chromosome size rank for the several cells of one plant and also of the variation between the plants of a species. It was found that within any one plant the variation between each of the series of measurements for corresponding size ranks was usually no more than .2 μ, at most .4 μ. The variation between corresponding plant averages and the species average was usually no more than .1 μ, at most .2 μ. It is felt therefore that reliance can be placed on the measurements obtained.

Cytological Observations

The chromosome numbers of these species were determined as *C. undulatum* (Nutt.) Spreng. *f. undulatum* and *C. undulatum f. album* Farwell, $2n=26$; *C. flodmanii* (Rydb.) Arthur *f. flodmanii* and *f. albiflorum* D. Löve, $2n=22$, in the following collections.

FIG. 12. Idiograms of chromosome complements of *C. flodmanii* and *C. undulatum*.

Cirsium undulatum (Nutt.) Spreng. 2n=26

CANADA—SASKATCHEWAN: Battle Creek Ranger Sta., Cypress Hills, July 25, 1947, *Breitung 5031*; 6 mi S. of Govenlock, July 29, 1953, *Senn 6204*; 10 mi E. of Maple Creek, July 14, 1950, *Dore & Breitung 11586*. ALBERTA: 1 mi E. of Lethbridge, Aug. 13, 1949, *Wagner 18*; Purple Springs, July 20, 1950, *Dore & Breitung 11850*; 2 mi E. of Lethbridge, Aug. 20, 1951, *Frankton 1342*; St. Mary's Dome, near Lethbridge, Apr. 9, 1949, *Frankton*

s.n. (seed of previous season only); 1 mi W. of Grassy Lake, Sept. 18, 1951, *Senn & Frankton 5902*. BRITISH COLUMBIA: 2 mi SE. of Hedley, Sept. 6, 1951, *Senn, Frankton & Gillet 5810*; 5 mi W. of Cascade, Sept. 5, 1951, *Senn, Frankton & Gillett 5765*; 10 mi NE. of Lytton, Sept. 12, 1951, *Senn & Frankton 5840*; 10 mi SSW. of Spences Bridge, on road to Lytton, July 12, 1953, *Calder & Savile 10397*. UNITED STATES—MONTANA: 4 mi S. of Brady, Aug. 22, 1951, *Senn, Frankton & Gillett 5648*; Meagher Co., 10 mi N. of White Sulphur Springs, Aug. 23, 1951, *Senn, Frankton & Gillett 5671*; Meagher Co., 10 mi N. of White Sulphur Springs, Aug. 23, 1951, *Senn, Frankton & Gillett 5672—f. album* Farwell. SOUTH DAKOTA: Custer Co., 20 mi SE. of Newcastle, Aug. 2, 1953, *Senn 6244*. IDAHO: Kootenai Co., 15 mi SE. of Coeur d'Alene, Sept. 4, 1951, *Senn, Frankton & Gillett 5757*.

Cirsium flodmanii (Rydb.) Arthur 2n=22

CANADA—ONTARIO: Port Arthur, Sept. 9, 1949, *Lindsay 203*. MANITOBA: La Salle, 15 mi SW. of Winnipeg, Aug. 18, 1948, *Dore & Johnson 9069*; Brandon, Aug. 24, 1949, *Stevenson F 131*; 10 mi E. of Dominion City, Aug. 24, 1948, *Dore 9211*; Swan River, Aug. 8, 1950, *Frankton 1197*; 6 mi W. of Letellier, July 22, 1953, *Senn 6185—f. albiflorum* D. Löve. SASKATCHEWAN: 11 mi E. of Hafford, July 19, 1949, *Frankton 961*; 3 mi S. of Franks Lake, July 22, 1949, *Frankton 986*; near Pike Lake, Aug. 1950, *Frankton 1387*; 4 mi E. of Saskatoon, Apr. 14, 1949, *Frankton s.n.* (seed of previous season only). ALBERTA: 2 mi SW. of Camrose, Aug. 7, 1950, *Frankton 1148*; Fort Vermilion, Aug. 20, 1946, *Groh 2819*. UNITED STATES—MONTANA: Cascade Co., 2 mi N. of Riceville, Aug. 23, 1951, *Senn, Frankton & Gillett 5657*; Carbon Co., 1 mi N. of Fremberg, Aug. 24, 1951, *Senn, Frankton & Gillett 5673* (unusually tall, 6 ft).

Chromosome Morphology

The karyotype of three plants of each species was studied. The chromosome size range and the total chromosome length for each of these is shown in Table II. Photographs and idiograms of the chromosome complements are shown in Figs. 6, 8, and 12, respectively.

The number of satellite chromosomes observed per cell was not constant and it is believed that under certain conditions the satellite is not visible as distinct from the body of the chromosome. The maximum number seen in one cell in the species *undulatum* was four; two on chromosomes of the longest

TABLE II

Chromosome size in *C. undulatum* and *C. flodmanii*

Species	Collection	Chromosome size range, μ	Total length, μ
undulatum	Breitung 5031	1.1–2.3	44.5
	Senn 6244	1.2–2.4	47.7
	Dore & Breitung 11586	1.1–2.3	44.8
flodmanii	Frankton, s.n.—Saskatoon, Sask.	1.5–2.6	44.1
	Stevenson, s.n.—Brandon, Man.	1.4–2.5	44.9
	Frankton 986	1.5–2.7	47.7

class, two on chromosomes of intermediate length. In *C. flodmanii* also the maximum number was four, all among the longest of the complement. The average total chromosome length of *undulatum* was 44.3 μ, of *flodmanii*, 44.2 μ. In general appearance, thickness, and staining reaction the chromosomes of both species are similar.

Pollen Characters

Pollen from dried specimens was examined in glycerin jelly mounts. As in all *Cirsium* species, the grains are tricolpate, the walls are thick and bear broad stout spines. The following measurements of the outer diameter include the spine tips. Considerable variation in diameter exists within a sample, usually of the order of 5 μ but occasionally as much as 10 μ. On the whole, pollen of *flodmanii* ranged from 40 to 58 μ in diameter, the averages of each sample falling in the range 42–55 μ. Grains of *undulatum* ranged from 52 to 65 μ in size, the sample averages varying from 56 to 60 μ. In general, pollen of *undulatum* is larger than that of *flodmanii* but there is considerable overlap in the ranges. Since the amount of chromatin in the chromosome complements of the two species is identical, the pollen size difference may not be correlated with the chromosome number difference but this, and the intraspecific variability, may be due to genic diversity between and within the species.

Discussion

It is well known that the basic chromosome number of the great majority of species of *Cirsium* is 17. Species from North America, Europe, and Asia have been found to be diploid; only two tetraploid Eurasiatic species (*C. vulgare, C. waldsteinii*) are known. In Japan, numerous polyploid (triploid, tetraploid, hexaploid) species have been reported by Aishima (1934), Arano (1957), and by other Japanese authors. The Far East is unique in the greater incidence of polyploidy and the occurrence of the hexaploid level.

A departure from the base number 17 was first reported by Ownbey (1951) for the two North American species *C. discolor* (Muhl. ex Willd.) Spreng. and *C. muticum* Michx., both $2n=20$. These counts have been confirmed in the present study, although not reported here, and two additional numbers of the non-17 series have been revealed.

It is significant that these four species are confined to North America. Each has an extensive range; their combined ranges cover almost the whole of the United States and southern Canada with the exception of the west coast of the continent. The species *muticum* and *discolor* occupy the eastern half of the continent, *undulatum* and *flodmanii*, the western half. Petrak (1917) placed the four species in the same subsection (Acanthophylla) in his monograph of North American species of *Cirsium* and their close affinity is recognized by the present authors.

The existence in North America alone of a few aneuploid species and the occurrence in both the New and Old World of numerous species of the base-17 series raises interesting speculation regarding the phylogeny of *Cirsium* and related genera. Two alternative explanations may be advanced for the existence of the two chromosome series: the genus may have originated with

the base number 17 and spread throughout the world in this condition, aneuploidy of the North American species arising later by reduction in number; *Cirsium* may be diphyletic, the aneuploid species arising from a stock similar to that from which *Carduus* developed and entering the genus *Cirsium* later by parallel mutation.

Cirsium is placed in the tribe Cynaroideae, subtribe Carduineae of Bentham and Hooker (1873); in the tribe Cynarae, subtribe Carduinae of Hoffman (1891). These systems agree on the majority of genera included in the subtribe: *Arctium*, *Carduus*, *Cirsium*, *Cousinia*, *Cynara*, *Goniocaulon*, *Jurinea*, *Myopordon*, *Saussurea*, and *Staehelina*. The genera *Berardia*, *Cnicus*, and *Warionia* of the Carduineae Benth. & Hook. are assigned to other subtribes by Hoffman, who groups with *Cirsium* the genera *Polytaxis* and *Lupsia*. Of the foregoing genera, chromosome numbers are recorded in the lists of Darlington and Wylie (1955) and of Cave (1956–58) for the following: *Arctium*, $x=8, 9$; *Carduus*, $x=8, 9, 11$; *Cousinia*, $x=13$; *Cnicus*, $x=11$; *Jurinea*, $x=15$; *Saussurea*, $x=9, 13$; *Cynara*, *Onopordon*, *Silybum*, *Warionia*, $x=17$. To these we can now add *Cirsium*, $x=10, 11, 13, 17$.

A pattern appears in the common occurrence of the number 17 as the sole or the highest base number of 5 of the 11 genera. A parallel process of reduction of numbers may have produced the similar series of numbers to be found in this group. The rarity or absence of the higher reduced numbers, 14, 15, 16, if not due solely to incomplete sampling, suggests that the reduction process is not recent in its inception. To explain the existence of the non-17 series by reduction in number is simpler than to postulate polyphyletic origins for these genera and it is believed that reduction has taken place independently in these stocks after they attained generic level.

Phylogenetic reduction of chromosome number in the Compositae is an accepted fact. Reduction is accomplished by translocation between nonhomologous chromosomes. Acentric segments are transferred from one chromosome to another, the former becoming shorter, the latter, longer. So long as a centric remnant has genetic value essential to viability, it will be retained as a chromosome. If, however, such a reduced chromosome lacks vital genes, it may be lost through meiotic accidents without detriment to the race. It seems possible that translocations may produce dicentric chromosomes. Difficulties at division will arise in such a case unless one of the centromeres becomes inactive. If such a reduction process has occurred in *Cirsium*, it may be expected that the complement of unreduced species ($x=17$) will consist of shorter, more uniform chromosomes than the sets of reduced species and that the total length of chromosomes of the latter may be equal to or slightly less than the total length of unreduced species. The amount of chromosome material lost in the process and the manner in which it is redistributed may be of interest.

The total length of the chromosomes of two representative North American species of somatic number 34 was found to be 43.8 μ, and 44.8 μ, of tetraploid *C. vulgare* ($2n=68$), 94 μ. With allowance for experimental error it appears that a 34-chromosome complement measures approximately 45 μ. The total lengths for the species *undulatum* and *flodmanii*, 44.3 μ and 44.2 μ respectively, indicate that phylogenetic reduction in number has resulted in very little or

no loss of chromosome material. The chromosomes of the unreduced species ranged in length from .9 to 2.3 μ, by small uniform steps. The still relatively continuous range of lengths of the chromosomes of *undulatum* and of *flodmanii* suggests that redistribution of the chromatin has been random.

The geographic distribution of *Cirsium* and related genera may be briefly mentioned. The Mediterranean region and adjacent western Asia are the areas common to the great majority of these plants. Most of the genera are rather small and of limited distribution in Eurasia. *Cirsium*, *Cousinia*, *Cnicus*, and *Saussurea* are the only genera of 100 or more species; only *Cirsium* and *Saussurea* occur in the New as well as the Old World.

So far as known, reduction of number in *Cirsium* has taken place only in a few North American species. The cause of the chromosomal instability of these species might provide interesting information on the evolutionary process. Several tentative explanations may be advanced: genes predisposing these species to chromosome breakage; unusual environmental conditions peculiar to North America in the past; difference in the age of species of the 17 and non-17 series.

It is obvious that to establish the present reduced karyotypes must have required a very great number of generations. Many intermediate numbers and karyotypes must have arisen and become extinct. The present karyotypes were found to be constant in the collections studied from several separated locations. The development of the reduced number and its spread over the present wide range of these four species points to a great antiquity for these species and their immediately ancestral stock in North America. The selective value of the chromatin redistribution is not apparent. The process has not altered the morphology beyond generic limits in *Cirsium* or in the related genera and certainly was not necessary to produce the great variability seen among the species of the x-17 series. Has the reduction in number and increase in size any intracellular advantage—facilitating mitotic or meiotic divisions, increasing or decreasing mutation rate or chromosome breakage? Rather it seems more probable that the reverse would be the case; that larger chromosomes would be more susceptible to meiotic accidents and to radiation-induced mutations. Redistribution of chromatin may even provide a mechanism by which duplicate genes may be dropped from a population. Acentric pieces of chromosome may be lost, in the course of these changes, without ill effect if they contained only genes which were present as well in the remaining intact chromosomes. In this way, duplicate segments acquired during evolution through wide hybridization may be removed from the genome. The net result of such a reduction process may be disadvantageous to the stock. Genetic incidents which, in unreduced species, may have, due to the presence of duplicate segments, produced viable mutants and potential new species, may, in reduced species, be lethal. However, measurements of chromosomes indicate that the loss of chromatin in the species *undulatum* and *flodmanii* has been slight and it is not believed that these species have yet become genically impoverished. It is anticipated that continuing work on this group may allow expansion of these philosophies and cast some light on the origin and migration of the genera.

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A PRELIMINARY STUDY OF DIFFERENTIAL POLLEN GRAIN PRESERVATION[1]

A. G. SANGSTER AND H. M. DALE

Abstract

Samples of pollen of three genera (*Populus*, *Pinus*, and *Typha*) which are normally shed at different times during the growing season were placed concurrently in four habitats (a pond, a lake, a swamp, and a bog). Their decomposition was traced through the summer and autumn of two seasons. The study shows that there is considerable variability in fossilization of pollen under identical conditions, and that the decomposition of the same pollen is dependent on the habitat, which affects not only the rate of decomposition but also the processes involved.

Introduction

By recording the presence of pollen grains or other distinctive plant remains preserved in the bog profiles, analysts have traced the succession of vegetation in a given area. The use of fossil pollen assumes that there is a distinctness in the grains and that the pollen grains are preserved in their original numerical proportion. It has been suggested that not all pollen grains are equally well preserved in a particular environment, and that conditions of preservation vary from one environment to another (3, 5, and 7).

The present study investigates preservation or decomposition of three types of pollen grains when subjected to the same field conditions, during the periods July to November in 1958 and 1959.

There were four sites used in this study. Site No. 1 was a 3- to 4-year-old farm pond dug on the grounds of the Ontario Agricultural College in 1955. It has a clay and gravel bottom overlaid with a thin layer of actively decomposing black organic matter. Species of *Chara* and *Spirogyra* were present in large quantities in the water, which was up to 7 meters deep, and *Typha latifolia* L. and *Alisma plantago-aquatica* L. grew at the edge (Fig. 4). Site No. 2 was a 10-acre lake, 3 miles west of Galt, Ontario, which was produced by the filling of a small depression left in a glacial moraine. The bottom was of loose flocculent marl and organic matter, unconsolidated to a depth of more than 4 meters. This shallow lake, almost filled with sediment, with a single inlet and a single outlet stream, contained heavy growth of both *Scirpus* spp. and *Nuphar advena* Ait. which extended up to 30 meters from shore. *Populus tremuloides* Michx. was a common species in the vegetation surrounding the lake (Fig. 5). Site No. 3 was a swamp located at the east end of Puslinch Lake, 12 miles SW. of Guelph. Under 15 cm of water, the soil was soft mud consisting of partially decomposed plant remains. *Typha latifolia*, *Salix* spp., *Utricularia* spp., *Nuphar advena*, and *Fraxinus nigra* Marsh. grew in the vicinity (Fig. 6). Site No. 4 was a mature 10-acre bog of *Sphagnum* hummocks

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interspersed with pools of water which dried up in the hot dry weather. Over the moss was a heavy cover of *Chamaedaphne calyculata* Moench., *Ledum groenlandicum* Oeder., *Vaccinium* spp., and in places a dense tree cover of *Larix laricina* Koch, *Picea mariana* B.S.P., and in some areas *Pinus strobus* L. and *Populus tremuloides* Michx. (Fig. 7).

TABLE I

Site	Daytime temp., °C	pH	Calcium ion conc., p.p.m.	Oxygen conc., p.p.m.
1	24.3	6–7	22.09	9.4
2	17.5	7	22.71	4.5
3	21.5	6	74.72	3.2
4	12.8	4–5	Negligible	Negligible

The water temperature at the height of summer varied with the site as indicated on Table I. Typical readings from site No. 4 were 10 to 12° C below those in the site No. 1, which heated to above 24° C. The pH of the bog water was only 4–5 whilst that of the other three sites was 6 to 7. The bog was low in calcium and sodium while site No. 3 with 75 p.p.m. calcium ions had more than three times the amount of sites No. 1 and No. 2. Oxygen was abundant in the farm pond but no significant reading could be obtained in the bog.

Method

The pollen chosen for this study was that of *Populus tremuloides* (poplar), *Typha latifolia* (cattail), and *Pinus strobus* (pine). These three pollen types are very distinctive in appearance, and are all released in great quantities but at different times during the growing season. *Populus* pollen is produced during the latter part of April or early in May in great abundance near the field sites but was not recognizable in surface sediment samples from these sites. Its absence from pollen spectra has been noted elsewhere (5). The grains (Fig. 8) are spheroidal, varying from 24 to 35 μ in diameter, with thick intines and extremely thin exines, reticulate or granular in appearance. The pollen of *Typha* was found in peat samples both from the surface and from a depth of 2 meters at site No. 4. It was present in surface samples at site No. 2, but not at a depth of 2.7 meters in the sediment although *Nuphar advena* (cow lily) pollen was found at both levels. *Typha* grains are released in great quantities during the first week in July (S. Ontario). It is estimated that each inflorescence of the closely related *Typha angustifolia* L. produces 174 million pollen grains per season (5). Figure 9 is a photomicrograph of a tetrad of *Typha* pollen grains. They remain united, usually in a square or rhomboidal arrangement. Each grain is from 18 to 23 μ in diameter and possesses a thick intine and thin, coarsely reticulated, exine. *Pinus* pollen is released in June in great quantities and is preserved very readily. It is a commonly reported species in the pollen spectra of bog and lake sediments of Eastern North America. The grains are characterized by two large bladders (Fig. 10) and vary in length

from 36 to 52 μ. The surface is covered by a very thick exine with a coarse, reticulate appearance which is expanded to form the two bladders.

In order to follow the course of decomposition, a study was undertaken of fresh and preserved pollen grains of each of the three types to show the variability in the appearance of the protoplasm and of the condition, thickness, and shape of the cell walls. Samples of older sediments of lake and bog taken with a soil auger were also studied to determine the state of the preservation of the same three species of pollen grains in the pollen spectra.

The pollen of each species for experimental work was collected and dried, without heat, in jars. To each was added a small amount of *p*-dichlorobenzene (moth crystals) and mold inhibitor (used for *Drosophila* culture). The jars were refrigerated. This treatment did not alter the appearance of the pollen grains.

Under natural field conditions pollen shed from the surrounding vegetation sinks through the water and comes to rest on the surface of the bottom sediment. The present study attempts to determine what happens to the pollen in the initial stages in the lake before it becomes covered over and incorporated into the sediment, which may take considerable time. Samples of the pollen were suspended at the surface of the sediment in small vials.

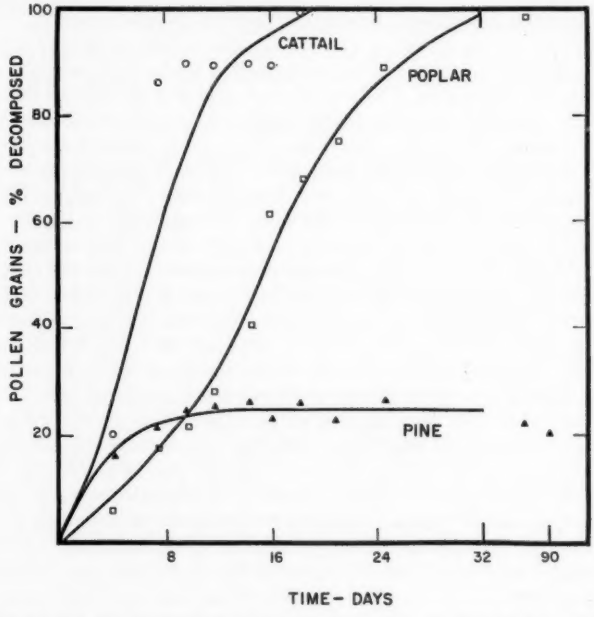
Early in July of 1958, twenty-five 50-ml vials, each containing a sample of a single type of pollen, were submerged side by side at each site in 15 to 20 cm of water. This was repeated in July 1959. The top of each vial was covered with protective nylon screening and was pushed down in the surface sediments at the bottom of the water. The top of each vial was level with the surface of the sediment, assuring that the pollen was in close association with the microhabitat. In the bog, due to dryness later in the season, these were pushed down in the peat and covered with wet *Sphagnum*. The vials were sampled when they were first placed in position and every third day for a period of 3 weeks, and then each month until November. Pollen to be examined was picked up in a tapered glass tube, randomly, at various levels of the sediment in the vials, ejected into a dilute formalin–aceto–alcohol solution, and refrigerated until examined. Each sample was centrifuged, and a small amount of pollen, which was concentrated in the upper layer of the centrifugate, was drawn into a fine pipette and added to a drop of water or glycerol on a microscope slide. The normal harsh chemical deflocculation of the sediment used in pollen analysis was unnecessary since the pollen grains were not consolidated.

By comparison with the original pollen the condition of 15 grains was estimated at 10 standard positions, using the co-ordinates of the mechanical stage. The condition of each grain was recorded giving a total of 150 grains per sample. For each species of pollen grain in a given environment, the samples of a complete series (12) taken from July to November were studied and counted in chronological order to measure the rate of initiation of disintegration. A magnification of 450 diameters was used for *T. latifolia* and *P. tremuloides*, and 100 diameters for *P. strobus*, in estimating the condition of the pollen grains. A grain was classified as faulty if there was any visible breakdown of either (or both) the cell contents and the exine that was obvious by comparison with the original pollen grains.

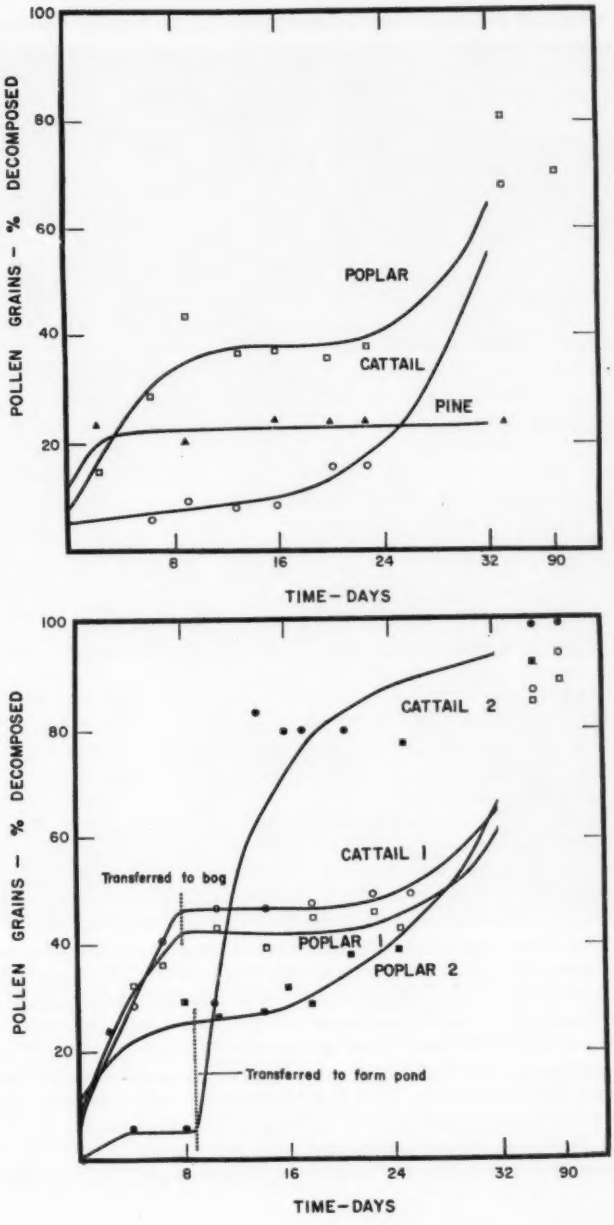
Characteristics of Decomposition in Each Environment

Populus tremuloides pollen decomposed at the same rate in both the farm pond (No. 1) and lake (No. 2), but at a slightly slower rate in the swamp (No. 3). After 32 days in Nos. 1 and 2 all grains were partially decomposed, the exine in many cases being affected as well as the protoplasm. In the bog, No. 4, the pollen was preserved in its original condition for 24 days; then degradative processes peculiar to this environment began to change the appearance of the protoplasts and alter the structure of the exines. After 32 days, 67% of the grains in the bog sample were affected, and 73% in the swamp, No. 3.

In all four environments, the decomposition of *Populus tremuloides* pollen grains progressed in the following manner: the protoplast either drew away from the walls to form a globular mass in the center, or it slowly disintegrated at various points throughout without gathering in the center, and, in each case, disappeared after 1 to 2 months. Accompanying the decomposition of the contents, the exine of most grains lost its spherical outline and gaps or breaks in its structure appeared (Fig. 8). As many as several hundred active bacteria were commonly seen inside single grains whose protoplast and exine were badly decomposed. The structural details of the green bacilli, which varied



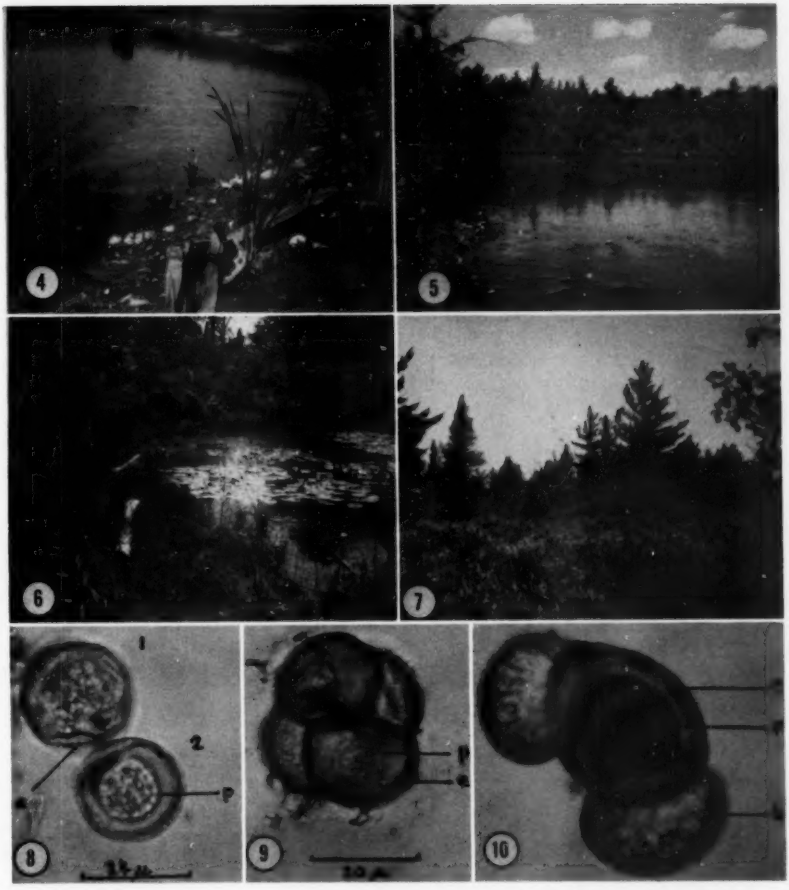
FIGS. 1, 2, and 3. The decomposition of the pollen during the first 32 days of the study. Figure 1 is the data for the farm pond. (Curves for the lake and swamp are very similar.) Figure 2 (p. 39, top) is the data for the bog. Figure 3 (p. 39, bottom) shows the data for the transfer experiments.


POLLEN GRAINS - % DECOMPOSED
100
80
60
40
20
POPLAR
CATTAIL
PINE
8
16
24
32
90
TIME - DAYS
CATTAIL 2
CATTAIL 1
Transferred to bog
POPLAR 1
POPLAR 2
Transferred to farm pond

in size, were not visible under oil immersion. Large numbers were present in the surface sediments of the farm pond, whereas only a few were seen in the surface sediment of the bog.

While approximately 90% of the pollen grains of *Populus tremuloides* were still recognizable, in all four environments after 4 months most of the exines were decomposed to thin, often broken shells. These are difficult to distinguish from the similar shells of other eroded pollen grains and from the other organic material of the sediment with which they become incorporated. By their appearance after one season, the exines of *Populus* pollen were in the process of being destroyed in all the environments on the surface of the sediment and this would explain why they were not found preserved in the pollen spectra of the older sediments of either the *Sphagnum* bog or the lake, although both were surrounded by a vegetation in which *Populus tremuloides* was a common species. Some remains of pollen grains from these trees must have been present in samples taken from the surface sediments of both the lake and the bog, but they were not identifiable after deflocculation with potassium hydroxide or by acetolysis. The rates of decomposition are indicated by the slopes of the curve in Fig. 1, similar curves could be drawn for sites Nos. 2 and 3 (the lake and swamp). Figure 2 demonstrates a somewhat different condition in the bog (site No. 4), in which the process is slower.

Typha latifolia pollen decomposed at a faster rate than did poplar pollen, in both the pond, No. 1, and the swamp, No. 3; at a similar rate in the lake, No. 2; and at a slightly slower rate in the bog, No. 4. All of the cattail grains were visibly affected after 16 days in site No. 1 and after 32 days in sites Nos. 2 and 3, while only 60% were affected after 32 days in the bog. An important difference during this initial phase was that, in the bog, only the contents of a grain decomposed, the exine being unaffected; whereas, in the other three sites (Nos. 1 to 3), the exines in addition to the contents of some grains showed evidence of breakdown. The decomposition in the other three sites proceeded as follows: the protoplasm drew away from the intine toward the center, where it formed a small globular clump which later disappeared, leaving the exine intact and fully expanded (Fig. 9). Complete collapse or decomposition of the walls accompanied the disappearance of the protoplasm in 10% of the grains in the pond and lake and in 20% in the swamp, during the time of the study. Globules were found adhering to the outside of the exine of many *T. latifolia* pollen grains in the farm pond, which, after investigation by microchemical tests, were concluded to be non-living material, probably oil, which had been exuded through the germ pore as a result of decomposition processes inside the grain or of immersion in the water (7). Gas bubbles and strong odors of decay were characteristic of the surface sediments of the farm pond and swamp and to a lesser extent, of the lake. Living bacteria were seen inside the protoplasts of only a few grains yet the contents of most grains had disappeared by the end of the study. In sites Nos. 1 and 2, approximately 90% of the exines (73% in site No. 3) of *T. latifolia* pollen grains were well preserved, in spite of the rapid decomposition which surrounded them, at the end of the study. However, since these grains were not found preserved in the older sediments of the lake bottom, the exines may eventually decompose.



FIGS. 4–7. Habitat pictures of pollen disintegration sites. Figure 4 shows *Typha latifolia* and *Alisma plantago-aquatica* growing at the edge of the farm pond in which masses of *Chara* and *Spirogyra* are floating. Figure 5 shows part of Altrieve Lake with *Nuphar advens* growing in shallow water. Figure 6 is of the swamp pool showing *Nuphar advena* and a heavy growth of *Salix* sp. in the background. Figure 7 shows a portion of the *Sphagnum* bog with *Pinus strobus*, *Larix laricina*, and *Populus tremuloides*. *Chamaedaphne calyculata* is prominent in the foreground.

FIGS. 8–10. Typical disintegrating pollen grains after 1 month in the surface sediments of the farm pond. Figure 8 shows pollen grains of *P. tremuloides* in which the exines, *e*, are partially decomposed, as well as the protoplast, *p*; the exine of the upper grain shows irregularity in its shape and the protoplast has partially decayed without drawing to the center as in the lower grain. Figure 9 shows a pollen tetrad of *T. latifolia* showing the protoplasts, *p*, drawn away from the walls. Figure 10 is a grain from *P. strobus*. The exines including the bladders, *b*, are well preserved.

The characteristics of an active decomposition made evident in the lake and pond by gas bubbles, bacteria, globules of oil, etc. were not present in the *Sphagnum* bog. The pollen grains of *T. latifolia* did not change in appearance during the first 20 days after they were placed in this environment, as indicated by curve on Fig. 2, then the protoplast drew away from the walls and formed a globular clump in the center which disappeared after several months, leaving the exine intact and well preserved. That they remain in this condition was shown by finding well-preserved exines of *T. latifolia* in samples of peat taken from a depth of 2.7 m in the bog. By the end of the study, 10% of the grains showed some evidence of wall collapse, but were still recognizable.

Pinus strobus pollen after 4 months in each environment showed no change in the thick exines (Fig. 10) but the contents of many grains had drawn away from the exine and formed a central, globular clump which must later disappear entirely, since it was absent from fossil *Pinus* pollen grains in samples of the older sediments of the lake and the *Sphagnum* bog. Some substance in the structure of these exines must have resisted the degradative processes to which they had been exposed. No bacteria were seen inside the protoplast of any *Pinus* pollen grain.

The Effect of a Bacteria Inhibitor

Bacteria occurred in decomposing *Populus* and *Typha* pollen grains in sites Nos. 1, 2, and 3. The effect of adding mercuric chloride to cut down the number of bacteria in the disintegrating *Typha* pollen grains was investigated. Pollen grains that had been in the farm pond for 9 days were brought into the laboratory and a solution of 1000 p.p.m. mercuric chloride added. Pollen grains were sampled at regular intervals and it was found that the rate of decomposition was slowed down, indicating that bacterial action is an important factor, but not the only one operating.

Transfer of Pollen from One Environment to Another

Pollen grains of the three species *Populus*, *Typha*, and *Pinus* were 'inoculated' by placing them in site No. 1 for a week, then they were transferred to site No. 4. The reciprocal process was also used by 'inoculating' pollen in site No. 4 for 9 days, then transferred to site No. 1.

Since there was no initial decomposition of the resistant pollen grains of *Pinus* in the pond (No. 1), there was no change when they were transferred to the bog and vice versa. *Populus* and *Typha* showed a rapid initial decomposition in the pond but this was inhibited in the bog for 20 days after transfer; then the new degradation process peculiar to the bog took over as indicated by the rising slopes of the curves in Fig. 3. Pollen of *Typha* when moved from the bog environment (No. 4) after 9 days began to decompose quickly in the pond, so that the bog had not conferred on the contents any additional resistance to decomposition. However, the poplar pollen showed no visible decomposition for the first 15 days after transfer from the bog to the pond, which may indicate that the bog medium had a slight fossilizing effect on these pollen grains which delayed the initiation of the decomposition process.

The Effect of an Oxidizing Agent

Using the Winkler method, samples of water surrounding the pollen grains indicated a higher oxygen concentration in the pond (9.4 p.p.m.), as compared to samples from the lake (4.5 p.p.m.) and the swamp (3.2 p.p.m.). Since oxidation might be an important factor in pollen disintegration, pollen grains of the three types were placed in a series of aqueous solutions of calcium hypochlorite of varying concentrations. These were sampled and studied after a period of 4 days. In a solution containing 2000 p.p.m. of chlorine, 93% of the *Populus* pollen grains were severely plasmolyzed. The colorless exines of all grains, which turned first yellow and then bleached white, lost their spherical shape, becoming irregular in outline or completely collapsed. Only 50% of *Typha* pollen grains were plasmolyzed. The contents had not become as reduced as in *Populus*, and none of the exines were bleached. *Pinus* pollen was neither plasmolyzed nor bleached. At 20,000 p.p.m. of chlorine, the contents and exines of poplar were so altered as to be unrecognizable. In *Typha*, all exines were bleached at 10,000 p.p.m., and 20,000 p.p.m. completely removed the reticulate pattern of the exine, leaving a thin hyaline layer, and destroyed the contents. The *Pinus* pollen exines were not all bleached until a concentration of 20,000 p.p.m. was reached, when the reticulation of the exine began to disappear and its brown color changed to white, leaving a brittle, hyaline shell which broke easily into fragments.

In the four environments used in this present study, aerobic decomposition was less severe than it was in the oxidizing chlorine solutions or than that experienced by pollen grains which fall into dry soil. At all sites the pollen grains were covered by water, which kept them in expanded condition and protected from the air.

Discussion

The processes of destruction or fossilization of pollen, seeds, and plant fragments which fall into bogs, swamps, and ponds has received little attention although stagnant water has been considered important as a preserving medium for plant remains. Bocock and Gilbert have studied the disappearance of leaf litter of various tree species in three different soils (2) and Barghoorn studied the biological, physical, and chemical changes which had occurred in the cells of fossilized plant remains in postglacial sediments and lignite (1). Erdtman, investigating the bogs of Alberta, found that while *Populus* spp. was an important plant in the surrounding vegetation and *Pinus banksiana* an insignificant one, yet 50% of the pollen spectra of these bogs consisted of pine whereas poplar left no record (5). Recently (4) it has been shown that the apparent frequency of pollen from tree species in the surface sediment of two ponds bears little quantitative relation to the basal area percentages of the same species in the surrounding vegetation. The pollen spectra of the surface sediments indicate 12% *Pinus* pollen and less than 1% *Populus*, whereas in the surrounding forest, samples showed 0.04% *Pinus* and 8% *Populus*. This has been interpreted as a difference between the representation of a species in pollen rain compared with the number of individuals of the same species in the surrounding vegetation. However, both *Populus tremuloides* and *Typha*

latifolia produce large quantities of pollen but are poorly represented in the pollen spectra of the surface sediments (4, 5).

This present study shows that there is a difference between the ability of pollen of various species to be preserved. This leads to a difference in the percentage frequency of species in the pollen rain spectrum and the fossil spectrum. Notably, poplar pollen grains disintegrate in bogs as well as elsewhere, and subsequent harsh chemical deflocculation of samples (e.g. oxidizing acids) renders the remaining fragments unrecognizable under the microscope. *Populus* is therefore underrepresented. Further study would indicate if all pollen grains could be placed in a series according to their tendency to disintegrate. Elsewhere it has been suggested that exines possess a material of extraordinary chemical resistance "sporopolenin" (6); presumably *Pinus*, well preserved in all four sites, is well endowed with this, while *Typha*, preserved with certainty only in the bog, occupies a middle position, and *Populus* is deficient since it disintegrated at all four sites.

It is clear that the processes in the bog, while perhaps different from those under the warmer, more aerobic, microbial conditions of the pond and lake, were also degradative with respect to the exines of poplar pollen grains. This fact, taken with the results of the transfer experiments and the general shape of curves showing the rates of degeneration of pollen grains, does not indicate that the bog environment or stagnant water, per se, will fossilize pollen grains, but suggests rather that before they ever enter the bog, or other similar medium, their ability to be fossilized, to a lesser or greater extent, is predetermined. The results of this study show that the resistance of the exine to an oxidizing agent is correlated with its ability to be fossilized.

There is an indication that the conditions at the surface of the sediments in the four sites used in this study vary in their ability to preserve certain species in the pollen rain. This suggests that the fossil pollen spectra from bogs may give a more complete and quantitatively accurate picture of the original pollen rain than do those from lake or pond sediments.

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SPECIATION AND BASIC CHROMOSOME NUMBER IN THE GENUS CELOSIA[1]

WILLIAM F. GRANT[2]

Abstract

A new allopolyploid species of *Celosia*, namely, *C. whiteii*, collected in Malaya and originating from a natural cross between *C. argentea* L. ($2n = 72$) and *C. cristata* L. ($2n = 36$), has been described and the somatic chromosome number determined as $2n = 108$. A somatic chromosome number of $2n = 18$ has been determined for another species of *Celosia*, namely, *C. trigyna* L. The basic chromosome number for the genus has been determined as nine. Of the four species of *Celosia* so far studied cytologically, each represents a different level of ploidy, from diploid ($2x$) to dodecaploid ($12x$).

Introduction

Of the total number of species of flowering plants which have been studied cytologically, it has been estimated that one-third are of polyploid origin (2). That speciation may occur as a result of a sudden change in the number of chromosomes is a well-known fact and many examples have been analyzed biosystematically (10; 11; 13, p. 308). Species originating through polyploidy have a high degree of interspecific reproductive isolation as a result of sterility barriers. Also, it has been strongly advocated that two or more individuals which may closely resemble one another but which are members of a polyploid series be given specific rank (8; 14). This paper concerns (1) the cytological and morphological observations on a collection of *Celosia* plants which indicate the presence of a new allopolyploid species derived from *C. argentea* L. and *C. cristata* L. and (2) the cytological observations on a second collection of *Celosia* plants, namely, *C. trigyna* L., which indicate that the basic chromosome number for the genus is nine.

Speciation

A natural interspecific hybrid between *Celosia argentea* ($2n = 72$), a pantropical herb (12), and cockscomb, *C. cristata* ($2n = 36$), a common garden ornamental found only under cultivation, has been reported by the author previously (3). From the few seeds produced by this nearly sterile hybrid a few plants were obtained, four of which were polyploids with somatic chromosome numbers of 108 (3, Table 2, p. 331; F_2 generation). All four were considered hexaploids, having apparently arisen through the union of nonreduced gametes (*AA* and *AABB*) and were classified as autoallohexaploids (genomic formula *AAAABB*; see 3, p. 333). The plants of hybrid origin to be reported on here grew from seeds of a single collection (Acc. No. 207) gathered by the author from one plant which seemed typical of a number

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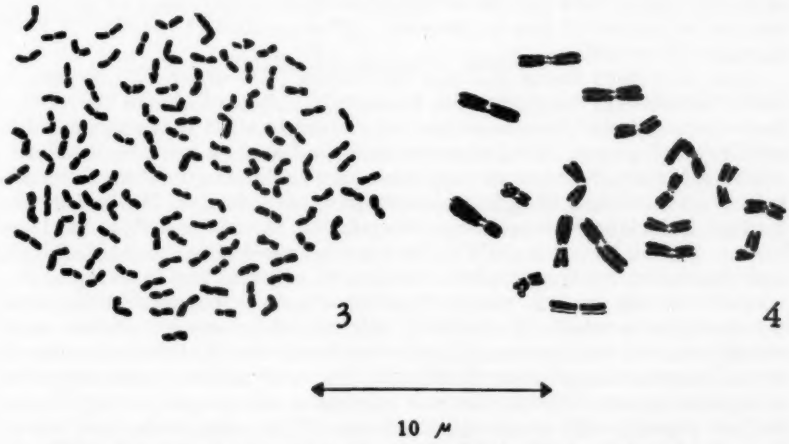
FIG. 1. Drawing of a young plant and a single mature spike of *Celosia whiteii* Grant. Plant × 2/3; spike × 3/4. [Drawing by Mr. J. A. Harshaw.]

FIG. 2. Photograph of an isotype of *Celosia whiteii* Grant.

growing in a cultivated garden near the 5.5 milestone, Klebang Road, Malacca, Malaya. Plants grown from these seeds, as well as the original plant, possessed spiked inflorescences characteristic of *C. argentea* (Figs. 1, 2) and the deep-red coloration in the stems and leaves similar to some deeply pigmented forms of *C. cristata*, giving a superficial resemblance to a non-fasciated form of this species. Cytological examination showed that each of these plants has a somatic chromosome number of 108 (Fig. 3). The chromosomes paired as bivalents, and behaved normally in both meiotic divisions. No supernumerary microspores were observed in the quartet stage and the pollen was of uniform size. Mature pollen grains, stained in fast green – lactophenol for an indication of fertility, gave 100% good pollen. This is in sharp contrast to the alloploids with this chromosome number derived from the natural hybrid previously reported in which meiotic irregularities were observed as well as supernumerary microspores, and the good pollen from the four plants was estimated to be 11, 30, 60, and 68%, respectively (3, Table 1, p. 328). In these latter plants, the presence of four homologous chromosomes of the *A* genome was considered to be the most likely cause of the irregular meiotic pairing and the subsequent pollen abortion. Since no cytological irregularities were observed in the allopolyploid plants of Malayan parentage, it would appear that the homologous chromosomes represented by the *A* genome in these individuals had become differentiated so that they were essentially non-homologous—presumably through natural selection of plants for normal fertility.

Since pollen grain size has been found to be a valuable criterion for the detection of polyploid plants in *Celosia*, and seems to be a diagnostic character for polyploid members of the Amaranthaceae (4), measurements were made of mature pollen grains from a plant of Malayan parentage (Acc. No. 207, $2n = 108$) and also from a plant of *C. cristata* (Acc. No. 30, $2n = 36$) for



FIGS. 3–4. Somatic metaphase chromosomes. Magnification ca. 3850×.
Fig. 3. *C. whiteii* Grant, $2n = 108$. Fig. 4. *C. trigyna* L., $2n = 18$.

comparison. The mean diameters of 20 measurements of pollen grains from these two plants were 36.1 μ and 26.2 μ, respectively.

Just how widespread these allopolyploids are at the present time is unknown. It might be expected, however, that they would become widespread, both through their fertility and their aesthetic qualities. Since there is no barrier to reproduction, these plants of hybrid and polyploid origin would appear to have become an established species which in time might become a less readily analyzable entity than at present. Accordingly this allopolyploid is here described as a new species.

Celosia whiteii *Grant, sp. nov.*

Herba annua ad 12 dm alta; culmus erectus, ruber, in statu sicco virescens, glaber, tum maturus multo ramosus; folia 4–15 cm longa, 0.5–4.0 lata, brevepetiolata, lanceolata, acuminata, attenuata, integra, rubra, glabrata, super lucida, subtus non lucida et intensius colorata; folia axillaria 5 aut minus, 2.5 cm longa, aut minus; inflorescentia dense spicata, dense cylindrica ad summis ramulis, 2–20 cm longa, 1.5–2 cm diam.; sepala 6–9 mm longa, lanceolatooblonga, ima parte spicae (7–14 cm) argenteo-alba aut pallide rosea, summa (7–14 cm) rosea aut rubra; stylus induratus maturus exsertus, roseus aut ruber quoque; semina 1.5 mm diam.; pollen circa 36.1 (34.4–37.7) μ diam.; $2n = 108$.

An annual herb, up to 12 dm high; stem erect, red, fading to green in dried specimens, glabrous, often much branched in mature plant; leaf-blades 4–15 cm long, 0.5–4.0 cm wide, short petiolate, lanceolate, acuminate, attenuate, entire, red, glabrous, above glossy, below non-glossy, coloration more prominent; axillary leaves 5 or less, 2.5 cm long or less; inflorescence a dense cylindrical spike terminating the branches, 2–20 cm long, 1.5–2 cm in diameter; sepals 6–9 mm long, lance-oblong, basal 7–14 cm of spike silvery-white or light pink, apical 7–14 cm pink or red; style indurate and exsert at maturity, pink or red; seeds 1.5 mm in diameter; pollen grains 36.1 (34.4–37.7) μ in diameter; $2n = 108$.

Type: In United States National Herbarium (US) *Grant 207*, August 5, 1957. Cultivated in the greenhouse, Macdonald College of McGill University, from seeds collected by the author from a single plant characteristic of a number growing in a cultivated garden near the 5.5 milestone, Klebang Road, Malacca, Malaya. Isotypes are deposited at the herbaria of the Plant Research Institute, Department of Agriculture, Ottawa (DAO); Herbier Marie-Victorin, Institut Botanique, Université de Montréal (MT); and the Wiegand Herbarium, Cornell University (CU). The type is named after Orland E. White, who discovered a natural hybrid between *C. argentea* and *C. cristata* (3).

It is now known that many allopolyploids derived from hybridization between species which have widely different chromosome numbers may resemble one of their parental species so closely that the differences seem negligible to a non-specialist. Such is the case of *C. whiteii*, which resembles *C. argentea* closely, differing only in the more pronounced axillary leaves and the pigmentation of the apical portion of the spike, stem, and leaves characteristic of *C. cristata*, and in the larger seed size (1.5 mm against 1.2 mm) and larger pollen grain size (36.1 μ against 31.1 μ). In *C. whiteii* it was observed

that the intensity of deep-red coloration of plants increased as exposure to direct sunlight increased. In contrast, the allopolyploids reported on earlier (3) had green stems and leaves and lacked pigmentation in these structures but retained the red pigmentation in the apical portion of the spike. Since differences in pigmentation in species of *Amaranthus* have been shown to be under genic control (5,9) it might be expected that red pigmentation of the stem and leaves may sometimes be absent in *C. whiteii*, thus leaving the red pigmentation of the apical portion of the spike as the chief macroscopical distinguishing characteristic. It was also observed that *C. whiteii* matured later than both *C. argentea* and *C. cristata*. This may be attributed to its higher ploidy and is characteristic of many higher members of a polyploid series (13). At Macdonald College (latitude approximately 45° 30′), *C. whiteii* did not flower out-of-doors during the normal growing period and it was necessary to bring the plants into the greenhouse in order for them to flower and produce seed. *C. whiteii* also differs from *C. cristata* in having the non-fasciated inflorescence, and a larger seed size (1.5 mm against 1.0 mm) and larger pollen grains (36.1 μ against 26.2 μ). The original source of the Malayan plants of *C. whiteii* which were grown and cared for by a native gardener was unknown. It seems probable, however, that his seeds came from plants grown in the vicinity. Accordingly, *C. whiteii* is considered to have originated through natural hybridization between *C. argentea*, which is reported as a common weed of cultivated fields in India, Burma, and the Malay Peninsula (1), and *C. cristata*, which is a common ornamental in the Malay Peninsula (6, 7), followed by doubling of the chromosome number. The restoration of fertility was not likely a single event for the cytological reasons given above, and in all likelihood required the differentiation of the different component chromosome sets so that they could function as in a diploid species. Thus it has been possible to detect at an early stage the method of speciation involved in the origin of this allopolyploid species.

Basic Chromosome Number

It is only when a cytological analysis of the majority of the representatives of a taxon has been carried out that a complete or nearly complete picture of the relationships of its members can be revealed with some certainty. Previously to this study, the chromosome numbers for only two species of *Celosia* (*C. argentea* and *C. cristata*) had been determined. A cytological study of another described species of *Celosia*, namely, *C. trigyna* L., enables one to consider that the basic chromosome number for the genus is nine.

A collection of seed (Acc. No. 341) of *Celosia trigyna*, a plant native to Africa, Madagascar, and Arabia, was obtained from the Botanic Garden (Hortus Botanicus Hauniensis), Copenhagen, Denmark. Herbarium specimens are deposited at the above-mentioned herbaria. The somatic chromosome number has been determined for several plants grown from this collection and found to be $2n=18$ (Fig. 4). This suggests that the basic chromosome number for the genus *Celosia* is nine. On this basis, *C. trigyna* ($2n=18$) is a diploid, *C. cristata* ($2n=36$) a tetraploid, *C. argentea* ($2n=72$) an octoploid, and *C. whiteii* ($2n=108$) a dodecaploid (12-ploid). Thus *Celosia* is presently an

example of a genus possessing a series of polyploid species in which each species cytologically examined represents a different level of ploidy.

Acknowledgments

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CONTROL OF CHLOROPHYLL CONTENT BY DAYLENGTH IN LEAVES OF MARQUIS WHEAT[1]

D. J. C. FRIEND

Abstract

The maximal chlorophyll contents reached during the growth of each of the first three leaves of Marquis wheat increased linearly with daylength, when the plants were grown from germination under illumination of 1750 ft-c intensity for daily periods of 8, 16, or 24 hours. This effect of daylength was also seen when chlorophyll concentration was expressed on a unit leaf area or fresh weight basis, and was related to increased leaf thickness at the longer daylengths. The increased chlorophyll content and concentration under long daylengths could not be duplicated by extending short daylengths with low-intensity supplementary light. Previous experiments suggest that the control of chlorophyll content by daylength is through the control of leaf thickness by the total amount of daily energy.

The ratio of chlorophyll *a* to *b* in mature leaves did not vary with daylength. There was also no change in the maximal chlorophyll concentration of leaves mature at the time of floral initiation compared with those mature in the vegetative stage of development.

Introduction

During studies on the control of flowering by daylength, it was noted by several workers that leaf color varied according to the light regime under which the plants were grown. Chailakhyan (2) found that seedlings of the short-day plants soybean and millet were darker green when grown in short daylengths than when grown under long daylengths. Murneek (13) reported daylength-induced changes in xanthophyll and carotene contents of the three short-day plants cosmos, salvia, and *Soja*, but chlorophyll was little affected. The chlorophyll content increased with the progress of the plant towards flowering under both short and long days, reaching a maximum at the time of seed pod formation. Other work has produced three main suggestions, (*a*) that long-day plants have most chlorophyll under long days and short-day plants under short days, day-neutral plants being relatively unaffected by daylength, (*b*) that long-day plants have most chlorophyll under short days and short-day plants under long days, day-neutral plants being unaffected, (*c*) that there is no connection between the photoperiodic response type of the plant and the formation of chlorophyll under different daylengths. Table I lists a few of the observations in the literature under these different categories.

Some authors have suggested a causal connection between the changes in chlorophyll and other pigment content produced under different daylengths, and the onset of the reproductive phase (10, 17). Other work has suggested that the parallel between chlorophyll content and change to the reproductive state is probably fortuitous. Roberts (14), working with etiolated plants

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TABLE I

The effect of daylength on the chlorophyll concentration in leaves from plants of different photoperiodic types

Photoperiodic type	Unit of chlorophyll concentration	Stage of plant development in: LD	SD	Reference
A. Short-day plants forming most chlorophyll under short daylengths				
Soybean, 8 vars.	Leaf area	F*	F	10
Phaseolus vulgaris, 6 vars.		F	F	
B. Short-day plants forming most chlorophyll under long daylengths				
Soybean var. Harbin	Leaf fresh weight	V†	V	3
Japanese millet		V	V	
C. Long-day plants forming most chlorophyll under long daylengths				
Oats var. Poblana	Leaf fresh weight	V	V	
Triticum sativum var. Lutescens		V	V	3
Phaseolus vulgaris	Leaf area	F	F	
Triticum sativum		F	F	10
D. Long-day plants forming most chlorophyll under short daylengths				
Nicotiana longiflora		F	V	
Phaseolus multiflorus		F	V	18
Trifolium pratense		F	V	
E. Day-neutral plants forming an equal amount of chlorophyll in long or short daylengths				
Soybean var. Graugelbe Industrie soya, Dickmann	Leaf area	F	F	10
Phaseolus vulgaris, 3 vars.		F	F	
F. Day-neutral plants forming most chlorophyll under long daylengths				
Lycopersicon esculentum var. Indiana Baltimore	Leaf fresh weight, leaf area	F	F	19

*F—Plants flowering or with flower initials present.
†V—Plants vegetative.

obtained by using low day temperatures and high night temperatures, found little connection between pigment content and flowering response.

In most of this work the effect of 'photoperiod' (total period of illumination by high or low light intensity) cannot be separated from that of daylength (duration of high-intensity light) (see, for example, 15, 3). It cannot be decided whether the observed effects on chlorophyll content are truly photoperiodic (i.e. reactions that can take place under low light intensities, such as control flowering in many plants) or simply related to the total radiant energy supplied to the plant. A true photoperiodic control of chlorophyll content has been shown in tomato (19) where chlorosis was induced by supplementing a 15-hour high-light period by 9 hours low-intensity light. Withrow *et al.* (20) have found that the lag phase of chlorophyll formation in etiolated leaves of bean exposed to light can be removed by short exposures to low levels of energy. The red/far-red pigment system operative in many other photoperiodic responses appears also to influence chloroplast development. Clauss (4, 5),

using the short-day plant *Perilla* and the long-day plant *Hyoscyamus*, has shown that the chlorophyll content alters when the dark period is interrupted by a short light break of high intensity. Using both 48- and 72-hour dark periods he has shown that there is a marked periodicity in the effectiveness of the light interruption, agreeing with Bünning's theory of endogenous alternating phases (1). However, although the photoperiodic responses of the two plant types are known to be out of phase, the effect of light on chlorophyll content was the same in both the long- and the short-day plant, so that the changes in pigment content cannot be causally related to flowering.

A recent paper by Chailakhyan and Bavrina (3) has attempted to reconcile these conflicting results by suggesting that studies on chlorophyll accumulation should take account of two stages of plant development. The first stage is before floral initiation, in which the chlorophyll content either increases or decreases according to the species or variety. Examples are the short-day plants whose contents decrease (millet) or increase (*Perilla*) just before the time of initiation, and the long-day plants whose chlorophyll contents decrease (*Rudbeckia*) or increase (wheat, bean) at this time. The second stage, that of flower formation, is accompanied by an increase in chlorophyll content in both long-day plants and short-day plants.

In the course of experiments on the effect of light intensity, temperature, and photoperiod on the growth and development of Marquis wheat, considerable information was collected on chlorophyll accumulation in the first three leaves on the main shoot. A further set of experiments were undertaken to determine whether the control of leaf chlorophyll content by daylength operated through a high light intensity process or through the action of low-intensity light such as is effective in promoting floral initiation.

Experimental Methods

(a) Growth Conditions

Seed of Marquis wheat (foundation stock 1956, Central Experimental Farm, Ottawa) was selected for uniform size, soaked in tap water at 23° C for 6 hours, plated onto moist filter paper in Petri dishes, and germinated at 23° C for a further 18 hours. The coleoptile had then emerged and seed could be selected for uniformity of germination. Eight seeds were sown in quartz sand in glazed 7-in. pots, and after 1 to 2 weeks' growth the number of seedlings was reduced to four.

The quartz sand was subirrigated four times daily with Hoagland's No. 1 nutrient solution modified by the use of "Sequestrene" (sodium ferric ethylenediamine tetraacetate), to supply 10 p.p.m. iron. The solution was maintained at a pH close to 5.6 and renewed at weekly intervals. Under these conditions, Hoagland's full strength solution gave significantly greater increases in the dry weight of the plants than did a well-fertilized potting soil.

The plants were grown in controlled environment rooms at a light intensity of 1750 ft-c (.096 g-cal (min cm^2)$^{-1}$ or 6720 μw cm^{-2} in the waveband 400–720 mμ) using General Electric standard cool white fluorescent tubes supplemented with 30 ft-c of incandescent light at the level of the top of the plants. It has been found in preliminary experiments that this level of incandescent

light was necessary to provide a photoperiodic response on flowering close to the optimal. The temperature was maintained constant at 20° C and the relative humidity was held at 70%. Further details of growing conditions are given elsewhere (8).

(b) Chlorophyll Determination

Measurements of chlorophyll content were made at 2- to 3- day intervals for the first, second, and third leaf on the main axis. In most experiments plants were sampled from the time of emergence of the first leaf until the senescence of the third leaf. For each leaf position, an extract was made from four leaves, and replicated three times, corresponding to four plants from three separate pots on each sampling occasion.

Leaf material was ground with 85% acetone with the addition of .02% sodium carbonate to prevent chlorophyll breakdown. The material was further extracted until colorless, and the extract was centrifuged to clear away plant debris.

Absorption coefficients of extracts were measured in a Beckmann DU spectrophotometer at a wavelength of 663 mμ and 645 mμ. The concentration of chlorophyll *a* and *b* was calculated using the specific absorption coefficients of MacKinney for 80% acetone (11). In cases where only the total chlorophyll content ($a + b$) was required, the absorption was measured at 663 mμ in a Spectronic 20 colorimeter. The concentration of total chlorophyll was then obtained from a calibration curve of absorption plotted against total chlorophyll content as determined by the Beckmann spectrophotometer. Further details of method are given in a previous publication (8).

The Constancy of the Ratio of Chlorophyll *a* to *b* under Different Daylengths

The purpose of this experiment was to determine whether the ratio of chlorophyll *a* to *b* in Marquis wheat changes with daylength, as reported in other plants (strawberry (16) and *Amaranthus* (4)).

The three daylengths studied were of 8, 16, and 24 hours' duration. In order to avoid error caused by periodic changes in the chlorophyll content during various phases of the light period (12), leaves were always sampled 1 hour before the end of the light period in the 16- and 8-hour daylength treatments. Samples of second leaves were taken daily from the 13th to the 26th day from soaking the seed. On day 13 the plants grown under the 24- and 16-hour daylengths had three leaves emerged, and the second leaf was about two-thirds of its final area. The plants under the 8-hour daylength had the third leaf just emerging, and their second leaf was about half its final area.

Results

There were no definite changes in the *a* to *b* ratio with time for any of the daylength treatments. The ratio was compared between the three different treatments at the time of their maximum chlorophyll content, and 1 day before and 1 day after this time. The results of an analysis of variance (Table II) show that the ratio was not significantly altered by daylength treatment, or by sampling occasion, and had a mean value of 2.9.

TABLE II

The ratio of chlorophyll *a* to *b* under different daylengths in the second leaf of Marquis wheat. Plants grown at a light intensity of 1750 ft-c and a temperature of 20° C. Each ratio is derived from measurements on 12 leaves

Daylength, hours per day	Ratio chlorophyll *a* to *b*			
	1 day before maximum*	Time of maximum	1 day after maximum	Mean
8	3.0	2.9	3.0	2.99
16	2.9	2.7	2.8	2.87
24	3.0	2.9	3.2	3.01
Mean	2.95	2.81	3.02	2.93

NOTE: No significant differences at the 5% level in the *F* test shown by an analysis of variance, for the effects of daylength or time.
*Maximum total chlorophyll content per leaf.

The Effect of Daylength on Chlorophyll Accumulation

The accumulation of chlorophyll was followed in the first, second, and third leaf of Marquis wheat plants under 8-, 16-, and 24-hour daylengths at a constant temperature of 20° C. The whole series of experiments was repeated under conditions of alternating temperatures, in which a period of 8 hours at 10° C was given at all three daylengths. In the 16-hour series this low temperature coincided with the dark period, in the 8-hour series, with the 8 hours' darkness, preceding the light period. On each sampling occasion, leaf area was measured by a photoelectric planimeter (6) and leaf thickness by a micrometer adjusted so as not to crush the leaves. Percentage transmittance of the chlorophyll extracts was measured in a Spectronic 20 photocolorimeter at 663 mμ. From the results of the previous experiments it was assumed that the ratio of chlorophyll *a* to *b* did not alter under the different daylength treatments, and total chlorophyll content ($a + b$) was calculated from the absorption at 663 mμ.

Results

In both temperature series the chlorophyll content in the first three leaves on the main shoot measured as mg per leaf or per unit leaf area successively increased with time. Data for the second leaf under constant temperature conditions is presented in Fig. 1, and the shape of the curve of chlorophyll content with time may be taken as typical, increasing up to a maximal value and then declining. Under any one set of environmental conditions this maximal chlorophyll content was reached successively later in the first, second, and third leaves. There was not sufficient data for fitting a regression of chlorophyll content with time for each leaf position under each environmental condition, and so obtaining a measure of the effect of daylength, leaf position, and temperature on the rate of chlorophyll accumulation. Also, because of differences in the rate of leaf development according to leaf position, temperature, and daylength, the chlorophyll contents at any one time are not comparable between different treatments. Accordingly, the effect of daylength, temperature and leaf position on chlorophyll content has been assessed from a series of

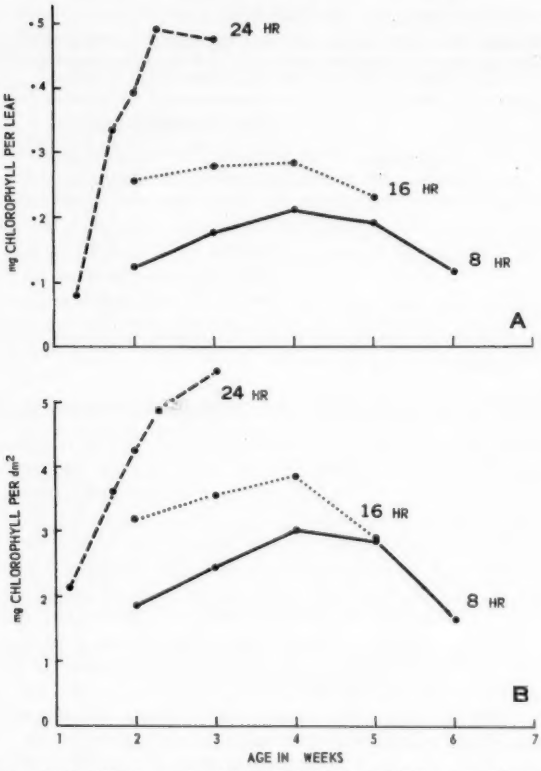

FIG. 1. The relationship between chlorophyll content of the second leaf of Marquis wheat and age of leaf, as affected by daylength. Each point represents a mean value derived from 12 leaves.

graphs similar to those in Fig. 1, by comparing the *maximal* chlorophyll contents reached during growth under any given set of environmental conditions. In the rest of this paper, the term 'chlorophyll content' refers to the maximal chlorophyll content developed under that particular treatment.

The data was subjected to an analysis of variance. In the alternating temperature series the 16- and 8-hour experiments were repeated, together with two additional experiments on the effect of low-intensity light, which will be discussed later. This data was then analyzed as shown in Table III, and the standard errors per treatment were calculated. The first replicate series gave lower maximal values than the second replicate series, but only in two cases did the replicates differ significantly in Duncan's test (7). The reasons for these differences are not known. The means of these replicate experiments under alternating conditions were then analyzed together with the data from the experiments carried out at constant temperature.

TABLE III

Agreement between replicate experiments on the effect of daylength on maximal chlorophyll content in Marquis wheat grown under alternating temperature conditions (16 hours at 20° C, 8 hours at 10° C). The values give the chlorophyll content in mg per leaf, each being derived from measurements on 12 leaves

	Daylength				
	Hours high-intensity light		Plus supplementary low-intensity light		
Leaf position	16	8	8 low plus 8 high	8 high plus 8 low	S.E.
Replicate series 1					
1	.22	.08	.15	.14	±.017
2	.35	.19	.19	.17	±.017
3	.57	.31	.31	.33	±.017
Mean	.378	.194*	.219	.211*	±.010
Replicate series 2					
1	.22	.13	.14	.14	±.017
2	.34	.25	.22	.24	±.017
3	.59	.40	.39	.39	±.017
Mean	.383	.258*	.249	.256*	±.010

*The first series differs significantly from the second series at the 5% level in Duncan's test (7).

TABLE IV

Effect of daylength, and of extending the daylength with low intensity light, on the maximal chlorophyll content of the first three leaves of Marquis wheat, grown under constant and alternating temperature conditions. The values give the chlorophyll content in mg per leaf, each being derived from measurements on 12 leave

		A Daylength hours high-intensity light			B Daylength supplemented by low-intensity light		
Temperature	Leaf position	24	16	8	8 hr. low + 8 hr. high	8 hr. high + 8 hr. low	S.E.
Constant, 20° C	1	.33	.21	.14	.11	.16	±.018
	2	.50	.28	.21	.21	.21	±.018
	3	.71	.61	.35	.39	.38	±.018
	Mean	.51	.37	.23	.23	.25	±.010
Alternating, 16 hr 20° C 8 hr 10° C	1	.30†	.22*	.11*	.14*	.14*	±.012
	2	.42†	.35	.22	.20	.20	±.012
	3	.66†	.58	.36	.36	.36	±.012
	Mean	.46‡	.38	.23	.23	.23	±.007

*Means of two replicate experiments, values derived from measurements on 24 leaves.
†S.E. = ± .018.
‡S.E. = ± .010.

In each variant the chlorophyll content, expressed as mg per leaf, was successively higher in the first, second, and third leaves on the main shoot (Table IVA). Comparing the means for the three leaf positions, it can be seen

that the chlorophyll content increased with daylength in both temperature series. There was no significant difference in chlorophyll content between the two temperature series.

The Effect of Extending Daylength by Low-Intensity Light

Experiments were carried out under both constant and alternating temperatures to determine whether extending an 8-hour daylength to a photoperiod of 16 hours with supplementary low-intensity illumination would give a similar chlorophyll content to that of plants under a daylength of 16 hours high light intensity. Incandescent light of 30–40 ft-c intensity at plant level was given for 8 hours either before or after the 8-hour period of high light intensity.

Results

In Table IVB are combined data from two replicate daylength series under alternating temperature conditions (see Table III) and one experimental series under constant temperature conditions.

As before, the chlorophyll content was successively higher in the first,

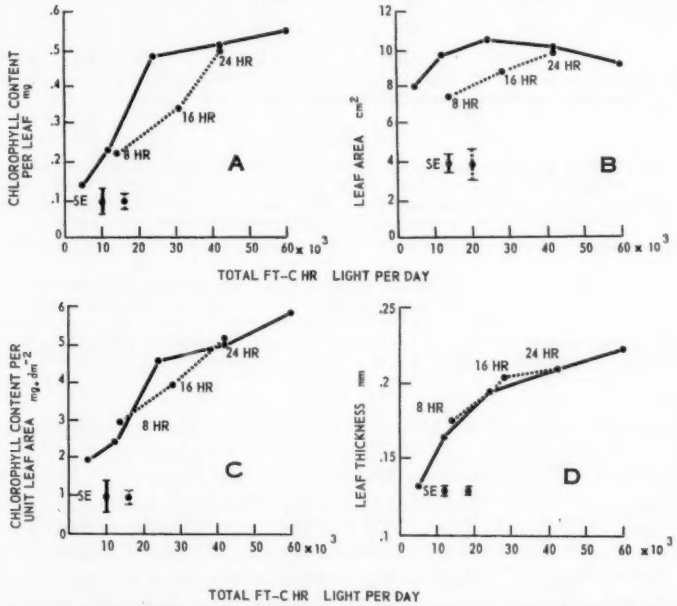

FIG. 2. Comparison of the effect of total daily light energy on chlorophyll content when given at a light intensity of 1750 ft-c for different daylengths (— — —) with the effect when energy was given under a 24-hour daylength at different light intensitites (———). Points are the mean of the three leaf positions, and are the mean of values from 36 leaves.

A. Maximal chlorophyll content, mg per leaf.

B. Leaf area, dm^2, at this time of maximal chlorophyll content.

C. Chlorophyll concentration per unit area, at this time.

D. Leaf thickness, mm, at this time.

second, and third leaves, and there was no significant difference between the chlorophyll contents formed under the two temperature treatments. The treatments given 8 hours' high-intensity light plus 8 hours' low-intensity light did not differ from those given 8 hours' high-intensity light alone. The increases in chlorophyll content produced by extending the daylength from 8 to 16 hours with high-intensity light cannot be obtained by extending the 8 hours of high-intensity light to a photoperiod of 16 hours by the addition of 8 hours of low-intensity light.

The Effect of Daylength and Photoperiod on the Development of Marquis Wheat

The developmental stage of plants was determined at each harvest by dissection of the apical meristem and examination under the microscope. A method of expressing the stage of development numerically, as a 'score', was modified from that used by Gregory and Purvis for rye (9). A score unit equals the number of days taken by plants of Marquis wheat, grown at a light intensity of 2500 ft-c, a daylength of 24 hours, and a temperature of 30° C, to reach certain morphologically recognizable stages of development. The rate of development is most rapid under these conditions, over a wide range of light intensities and temperatures studied. As the progress of score with time is linear for short periods under most environmental conditions, an arithmetic mean score can be obtained from the range of morphological stages present in any one group of plants.

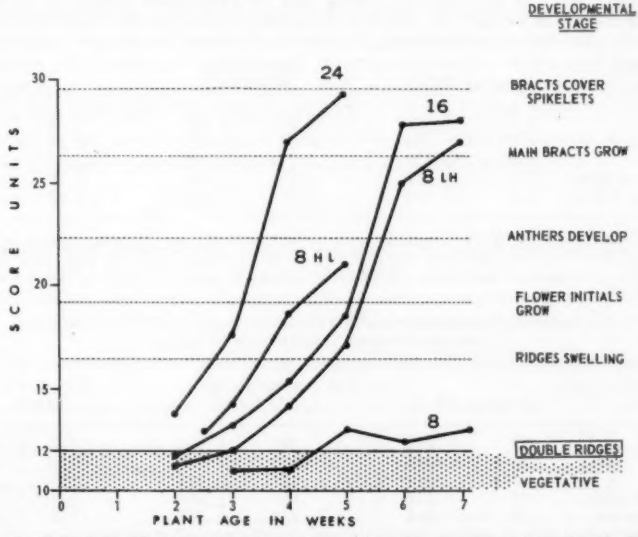

FIG. 3. The effect of daylength and photoperiod on the progress to flowering in Marquis wheat. Each point is a mean value derived from 10 plants. For further descriptions of developmental stages, see ref. 9.

Results

The rate of development of plants grown at a temperature of 20° C under the five light treatments is shown in Fig. 3. Wheat being a long-day plant, the score increased more rapidly under a 24-hour daylength than under a 16- or 8-hour daylength, and floral initiation, as shown by the appearance of double ridges on the stem apex, was progressively earlier the longer the daylength. When the photoperiod was extended from 8 to 16 hours by supplementing the main light period by 8 hours of low-intensity light (treatments 8 HL and 8 LH in Fig. 3), the score was higher at all times than that in the treatment given 8 hours' high-intensity light alone, and was close to that of the treatment given a daylength of 16 hours' high-intensity light.

Discussion

The differences in chlorophyll content (mg per leaf) between the first, second, and third leaves on the main shoot of plants grown under any one set of conditions can largely be attributed to the greater area or fresh weight of the older leaves. When chlorophyll is expressed on a unit leaf area or fresh weight basis, differences between the first three leaves are greatly reduced (Table VA) or not significant (Table VB).

TABLE V

Effect of daylength on the maximal chlorophyll concentration in the first three leaves of Marquis wheat, under constant and alternating temperature conditions

A. Chlorophyll per unit leaf area. The values give the chlorophyll content in mg per dm^2, and are means for leaf 1, 2, and 3, and represent measurements from 36 leaves

Daylength hours	Constant 20° C		Alternating 20°/10° C		Mean	
24	5.2	±.129	5.5	±.129	5.35	±.091
16	4.0	±.129	4.5*	±.149	4.25	±.091
8	3.1	±.129	2.8*	±.149	2.95	±.091
8 low plus 8 high	2.5	±.129	3.0*	±.149	2.75	±.091
8 high plus 8 low	3.0	±.129	2.9*	±.149	2.95	±.091

*Means of two separate experiments.
NOTE: *Effect of temperature:* constant 20° C, mean = 3 56 S.E. ± .058, alternating 20/10° C, mean = 3.82 S.E. ± .058.
Effect of leaf position: leaf 1, mean = 3.88 S.E. ± .070, leaf 2, mean = 3.56 S.E. ± .070, leaf 3, mean = 3.62 S.E. ± .070.

B. Chlorophyll, % fresh leaf weight. Values are means of leaf 1, 2, and 3 and represent measurements from 36 leaves

Daylength hours	Constant 20° C		Alternating 20°/10° C		Mean	
24	.29	±.014	.25	±.014	.274	±.010
16	.20	.014	.23*	±.008	.213	.010
8	.17	.014	.15*	±.008	.156	.010
8 low + 8 high	.13	.014	.16*	±.008	.147	.010
8 high + 8 low	.17	.014	.15*	±.008	.150	.010

*Means of two separate experiments, no significant difference between replicates.
NOTE: *Effect of temperature* not significant; constant 20° C, mean = .195, alternating 20/10° C, mean = .181.
Effect of leaf not significant; leaf 1, mean = .197, leaf 2, mean = .191, leaf 3, mean = .178.

Marquis wheat is a long-day plant and behaves in a similar manner to Lutescens wheat (3) in that most chlorophyll was formed under the longest daylength. This effect, shown by the linear increase with daylength of the maximal amount of chlorophyll accumulated in the first three leaves (Fig. 2A, 2B), was not caused by daylength-induced changes in leaf area or fresh weight. The daylength effect was still evident when chlorophyll content was based on leaf area or on leaf fresh weight (Table V, A and B, and Fig. 1B).

The chlorophyll content of Marquis wheat was not affected by photoperiod, as extending an 8-hour daylength to a photoperiod of 16 hours by the addition of 8 hours' low-intensity light had no effect on the chlorophyll content (Tables IV, VA, VB). An alternative possibility is that the differences in chlorophyll content in plants grown under different daylengths were related to the total amount of light energy received each day. Evidence in support of this view is provided by data from a previously reported experiment (8), in which plants were grown under the same conditions of nutrient supply, temperature, and humidity as used in the constant temperature series of experiments reported here. Illumination was provided by the same source, using a daylength of 24 hours at a range of 5 light intensities from 200 to 2500 ft-c. We can then compare the effect on chlorophyll contents of two different methods of providing the same total daily light energy. Data on the first method, in which a certain intensity of light was given for a 24-hour daylength, is provided by the previously reported experiment, with interpolation where necessary. Data on the second method, that of giving a higher light intensity for a daylength shorter than 24 hours, is provided in this present paper. A comparison has been made of the effect of total daily light energy, given in these two different ways, on the chlorophyll content expressed as mg per leaf (Fig. 2A). The content of plants given an 8- and 16-hour daylength was lower than that of plants given an equal total daily radiation under a 24-hour daylength. However, the leaf area was also lower (Fig. 2B) so that when chlorophyll was expressed on a unit leaf area basis (Fig. 2C) there was no significant difference between the two methods of supplying light energy.

TABLE VI

The developmental stage of the plant at the time of the maximal chlorophyll content in the first and third leaves of plants grown under different photoperiods at a constant temperature of 20° C

Daylength	Stage of development (score,* see Fig. 3)	
	At time of chlorophyll maxima in:	
	first leaf	third leaf
8 hours high light	Less than 12	13.0
8 hours high plus 8 hours low	Vegetative	12.0
8 hours low plus 8 hours high	13.1	21.1
16 hours high light	12.0	13.2
24 hours high light	13.7	17.6

NOTE: There were no significant differences in chlorophyll concentration measured as % fresh weight between leaf 1 and leaf 3 in any of these treatments as shown by Duncan's test (7).

*'Score' was derived by dissection of the same plants as were used in chlorophyll analysis. Each figure is the mean value from 12 plants.

The question remains as to the mechanism of the effect of daylength on the chlorophyll content per unit leaf area. Previous work (8) has shown that the increase in chlorophyll content with an increase in light intensity under a 24-hour daylength was related to an increase in leaf thickness. The same relationship between an increased chlorophyll content per unit leaf area and an increased thickness was also found in these present experiments. The correlation coefficient of chlorophyll per unit leaf area on leaf thickness was .82 (significant at the .05 level) for the constant temperature series, and .91 (significant at the .01 level) in the alternating temperature series. From Fig. 2D it can be seen that the leaf thickness is related to the total daily light energy, whether this light energy is given continuously or as a daylength shorter than 24 hours. It therefore appears that daylength controls chlorophyll content through the effect of total daily light energy on leaf thickness.

The view of Chailakhyan and Bavrina (3) that the chlorophyll concentration in leaves of wheat increased at the time of floral initiation is not supported by my experiments. There was no significant difference in the chlorophyll concentration measured as % fresh weight (Table VB) between the first leaf and the third leaf in any one treatment, although the score at the time of the maximal chlorophyll content in the third leaf was in all cases higher than that at the time of the maximal chlorophyll content in the first leaf (Table VI). The stage of development in the third leaf had passed the stage of floral initiation (score 12) whereas the stage of development in the first leaf was either in the vegetative phase or in the early stages of floral initiation.

In contrast to some other plants (e.g. strawberry (16)), mature leaves of wheat showed no significant alteration by daylength of the chlorophyll *a* to *b* ratio.

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COMPARATIVE RESPONSE OF TWO SAPROPHYTIC AND TWO PLANT PARASITIC SOIL FUNGI TO TEMPERATURE, HYDROGEN-ION CONCENTRATION, AND NUTRITIONAL FACTORS[1]

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Abstract

The behavior of two soil saprophytes, *Trichoderma viride* and *Trichocladium asperum*, and two root-infecting fungi, *Ophiobolus graminis* and *Fomes annosus*, was compared under various conditions in laboratory culture.

On an agar-solidified organic medium optimum temperatures for growth were approximately: *T. viride* 25–30° C, *T. asperum* 20–25° C, *O. graminis* 20–25° C, *F. annosus* 25° C. *T. viride* rapidly outgrew the other fungi in the optimum range but this relationship changed at lower temperatures, its growth rate being equalled by that of *O. graminis* at 10° C. *T. viride* was the only fungus to grow at 35° C. In a synthetic liquid medium adjusted to pH values from 3.0–7.0 with a citrate–phosphate buffer, growth of *O. graminis* and *F. annosus* was sharply reduced at pH values below 5.0. *T. viride* made good growth at pH 3.1 and reduction in growth of *T. asperum* occurred only below pH 4.0. Both parasites required thiamine for growth in a synthetic medium and *O. graminis* also required biotin; in addition they showed a preference for organic as opposed to inorganic nitrogen sources. *T. viride* and *T. asperum* grew well with KNO_3 as nitrogen source and neither required vitamins. D-Glucose, D-fructose, and D-mannose were readily utilized, and D-arabinose poorly utilized, by all four fungi. Utilization of other hexoses, pentoses, disaccharides, and polysaccharides varied considerably between the fungi.

The relationship of the results obtained to the observations of others on the ecology of soil fungi is discussed and the possibility that combinations of physical and nutritional factors may favor specific fungi in the soil is considered.

Introduction

The experiments described in this paper were designed to compare the growth of two saprophytic and two plant parasitic soil fungi under various conditions in laboratory culture. Information concerning the nutrient and other factors which influence the growth of such fungi should be of assistance in explaining their behavior in their soil habitats.

In order to colonize soil substrates fungi must be able to utilize them as sources of nutrients and they must be able to do so in competition with other members of the soil microflora. Garrett (10) introduced the term 'competitive saprophytic ability' to express differences in the facility with which fungi competitively colonize dead organic matter in the soil, and typical root-inhabiting fungi have been shown to have a lower competitive saprophytic ability than obligate soil saprophytes (2, 20, 23). However, fungi are likely to compete most strongly under conditions which favor them most and hence competitive saprophytic ability cannot be considered a constant characteristic but one that will vary, within limits, according to the environmental conditions. There is considerable evidence that nutritional factors pro-

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foundly influence the behavior of fungi in the soil. Stover (29), for example, has shown recently that the activity of *Fusarium oxysporum* f. *cubense* in the soil is altered appreciably by the addition of nutrients, and similar evidence is provided by numerous other investigations in which soil amendments have been used.

The results of the experiments to be described here indicate that the four fungi studied differ widely in their response to physical and nutritional factors and suggest that in their natural habitats they would react in different ways to similar environmental conditions.

Materials and Methods

Fungi Studied

Investigations of four fungi are reported in this paper, two soil saprophytes *Trichoderma viride* Pers. ex Fries and *Trichocladium asperum* Harz., and two plant parasites *Ophiobolus graminis* Sacc. and *Fomes annosus* Fries.

Trichoderma viride and *Trichocladium asperum* were isolated from a soil sample taken at a depth of 1 inch below the surface of a wheat field at Edmonton, Alberta. An isolate of *Ophiobolus graminis* was obtained from diseased wheat roots, and its pathogenicity was demonstrated by the production of typical "take all" symptoms in wheat seedlings grown in artificially infested soil. The culture of *Fomes annosus* was from the culture collection of the Forest Pathology Laboratory at Calgary, Alberta.

Stock cultures of the four fungi were grown at room temperature on slants of M.Y.G. agar (see below) in test tubes and stored at 0° C.

Media

A solid organic medium (M.Y.G. agar) was employed for the maintenance of stock cultures and the investigation of temperature response. All four fungi grew well on this medium, which was composed of 5 g malt extract (Difco), 5 g yeast extract (Difco), 15 g D-glucose, 17 g agar (Difco-Bacto), and 1 liter distilled water.

A synthetic medium was used in all other experiments. It had the following composition:

D-Glucose	12.51 g
l-Asparagine	2.36 g
KH_2PO_4	1.00 g
$MgSO_4.7H_2O$	0.50 g
$Fe_2(SO_4)_3$	0.715 mg
$ZnSO_4.H_2O$	0.540 mg
$MnCl_2.4H_2O$	.135 mg
Agar (Difco-Bacto) in solid media	17.0 g
Distilled water to make	1 liter

A vitamin supplement was included except where the effect of vitamins on growth was studied. It consisted of the following:

Thiamine hydrochloride	100 μg per ml
Pyridoxine hydrochloride	100 μg per ml
Calcium *d*-pantothenate	100 μg per ml
d-Biotin	10 μg per ml

i-Inositol	5 mg per ml
Niacinamide	100 μg per ml
p-Aminobenzoic acid	100 μg per ml

These solutions were made up in 20% ethyl alcohol and stored at 0° C. One milliliter of each was included in 1 liter of medium.

Glucose and asparagine were replaced by equivalent amounts of other carbon and nitrogen sources in certain experiments which will be described later.

The pH of the synthetic medium was adjusted to 5.5 with 0.5 *N* KOH before autoclaving, during which a change of not more than 0.1 of a pH unit occurred. Agar media were autoclaved in plugged test tubes containing 15-ml aliquots. These were then poured directly into Petri dishes ensuring equal quantities per dish. For liquid cultures 20 ml of medium were dispensed into 200-ml Erlenmeyer flasks. All media were sterilized by autoclaving for 8 minutes at 121° C.

Pyrex glassware was used throughout and it was cleaned with hot detergent solution followed by thorough rinsing with tap water and with distilled water. All chemicals were of the highest grade commercially available.

Experimental Conditions and Determination of Growth

Where possible, experiments were carried out using agar plates as these gave readily reproducible results and permitted the measurement of growth after different periods of incubation. Liquid media were employed for the study of pH response and carbohydrate utilization, and in these experiments total yield of mycelium only was determined. Growth rates on agar media containing different carbohydrates varied little and bore no relationship to the differences in utilization indicated from the results of liquid culture experiments. Brancato and Golding (1) have discussed the suitability of the agar plate method for carrying out certain types of experiments with fungi.

Inoculum in all cases consisted of plugs 5 mm in diameter cut from the periphery of colonies growing either on M.Y.G. agar or on the solidifed synthetic medium, to correspond with the medium of the experiment being undertaken. The importance of using inoculum from the same part of a colony was demonstrated by comparing growth from plugs cut from the periphery with that from plugs from an adjacent position along radii of colonies of both *O. graminis* and *F. annosus*. Although the final growth rate was the same, total growth was significantly less from plugs from the inner position.

In liquid cultures the inoculum plug was held at the surface of the medium by means of a glass rod with a bent tip as described by Converse (3). Highly variable yields were obtained otherwise. Submerged plugs in general gave inferior growth.

Except where otherwise indicated, cultures were incubated at room temperature (22–25° C) and received diffused daylight from a northern window.

The length of incubation is of considerable importance if yield only is determined as in the liquid culture experiments described below. This is particularly true where, as in this investigation, fungi differing widely in their growth rates are being compared and a uniform incubation period cannot be used. In an attempt to overcome this difficulty each fungus was considered

separately and in each experiment incubation was continued for no longer than was required for the mycelium to cover the surface of the most favorable medium.

Growth on solid media was determined as the mean diameter in millimeters of three or four replicate cultures. The mycelial mats from liquid cultures were harvested on previously dried and weighed filter papers, washed thoroughly with distilled water, and dried for 24 hours at 90° C. The mean dry weight was determined from four or five replicate cultures.

Experimental Results

Influence of Temperature on Growth

The growth rates of the four fungi were determined on M.Y.G. agar in Petri dishes at 10, 15, 20, 25, 30, and 35° C by measuring colony diameters of three replicate cultures at each temperature twice daily. Humidity was not controlled and this tended to be higher in the Petri dishes at the lower temperatures. The results obtained are indicated in Fig. 1.

It is apparent that there were appreciable differences in the temperature responses of the four fungi studied. For *T. viride* the optimum temperature was between 25 and 30° C and was higher than that of the other three fungi. *T. viride* was the only species which grew at all at 35° C, although this was probably close to a maximum, for growth ceased after 41 hours. It grew rapidly at 30° C, whereas the other three fungi grew only slowly at this temperature. At temperatures of 20° C and above the typical high growth rate of this fungus was clearly demonstrated, but at lower temperatures there was an appreciable lag before measurable growth occurred and at 10° C the growth rate was quite markedly reduced. Similar results were obtained by Ludwig (21) except that 40° C was found to be the maximum in his experiments.

The optimum temperature for *T. asperum* was in the region of 20° C, and the maximum was 30° C, although growth ceased after 41 hours at this temperature. This species grew comparatively well at 10 and 15° C, but even in the optimum range it grew slowly. Its growth rate was exceeded by that of *O. graminis* at all temperatures and by *F. annosus* at 25 and 30° C.

The maximum temperature for growth of *O. graminis* was 30° C, where growth was arrested after 48 hours. The optimum appeared to lie between 20 and 25° C and good growth occurred at 10 and 15° C. These results are in accord with those of Davis (5). At 10° C *O. graminis* grew as rapidly as *T. viride* and commenced growth after a shorter lag.

The isolate of *F. annosus* studied had a rather limited temperature range for growth. No growth took place above 30° C or below 20° C; the optimum temperature was 25° C. It is possible that if a longer incubation period had been employed growth might have been observed at lower temperatures. Rishbeth (26) observed growth to occur over the range 0° C to 28.5° C with optimum growth at 22.5° C. However, Humphrey and Siggers (15) found the over-all temperature range for growth of a European isolate of *F. annosus* to be much lower than that of a North American isolate, and it may be assumed that strain differences exist in this species.

Although the optimum temperatures for growth of all four fungi lie within a range of approximately 5 centigrade degrees, *T. viride* and, to a lesser extent, the *F. annosus* isolate are apparently better adapted to growth at higher temperatures whereas *O. graminis* and *T. asperum* are able to grow satisfactorily at much lower temperatures.

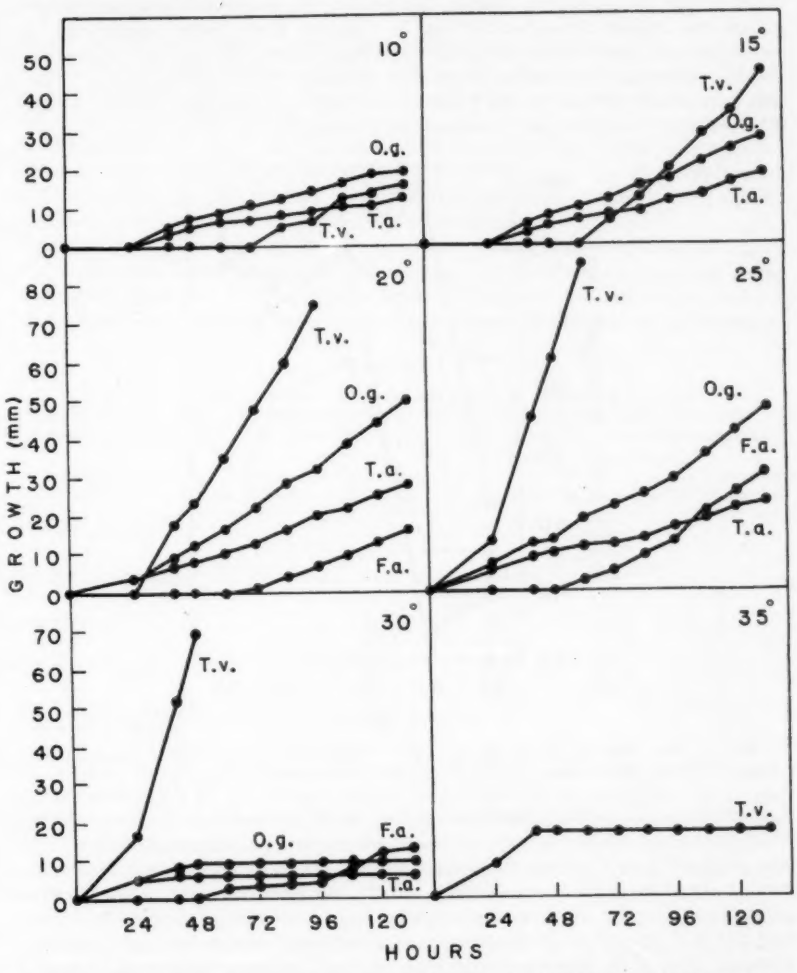

FIG. 1. The effect of temperature (°C) on the growth of *Trichoderma viride* (T.v.), *Trichocladium asperum* (T.a.), *Ophiobolus graminis* (O.g.), and *Fomes annosus* (F.a.) on M.Y.G. agar.

Influence of Hydrogen-ion Concentration on Growth

The pH of the liquid synthetic medium was adjusted to 3.0, 4.0, 5.0, 6.0, and 7.0 by means of a citrate–phosphate buffer. Various proportions of 0.1 *M* citric acid and 0.2 *M* K_2HPO_4 were mixed to give the required pH, and the mixtures were incorporated in the medium so as to constitute one third of the final volume. Slight changes in pH occurred during autoclaving giving the values indicated in Fig. 2. Culture flasks were inoculated, and incubated at room temperature as described previously and for each fungus the mycelium was harvested from four replicate flasks at each pH. *T. viride* was incubated for 6 days, the other three fungi were harvested after 17 days. The mean dry weights of mycelium are plotted in Fig. 2.

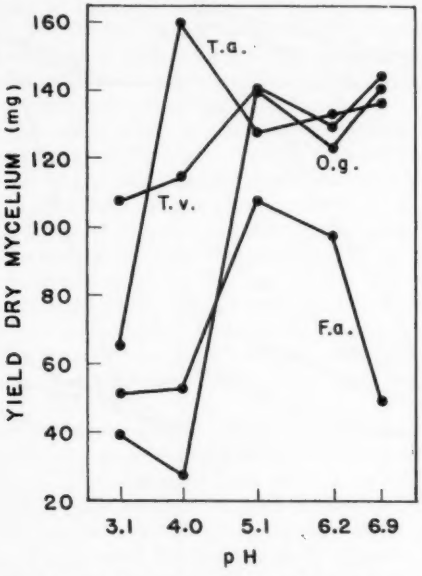

FIG. 2. The effect of pH on the growth of *Trichoderma viride* (T.v.), *Trichocladium asperum* (T.a.), *Ophiobolus graminis* (O.g.), and *Fomes annosus* (F.a.).

Except for *T. viride* growth appeared to be retarded and this may have been due to the buffer. Treschow (30) reported that phosphate used as a buffer was inhibitory to *Psalliota bispora* at similar concentrations. In spite of this, certain features stand out clearly. Both saprophytes were able to make good growth under more acid conditions than were the parasites. At pH 3.1 and pH 4.0 growth of *O. graminis* and *F. annosus* was considerably reduced whereas that of *T. viride* was still high at these values as also was that of *T. asperum* at pH 4.0. At higher pH values there was little difference in the yield of mycelium from *O. graminis* and from the two saprophytes, although the shorter incubation period of *T. viride* should not be overlooked. With

the pH range used it was not possible to determine the optimum pH or the upper limit for growth except for *F. annosus*. For the isolate studied here the optimum pH was 5.1 and the range probably did not extend much beyond pH 3.1 and 6.9. These values are similar to those obtained by Robak (28) for this fungus. The results obtained with *O. graminis* correspond in general to those reported by other investigators for similar pH values (5, 32).

Influence of Nitrogen Source and Vitamin Supplement on Growth

A series of experiments was carried out to compare growth on organic and inorganic nitrogen sources in the presence and absence of the vitamin supplement. Synthetic media lacking the vitamin supplement were prepared with KNO_3, $(NH_4)_2SO_4$, *l*-asparagine, or yeast hydrolyzate (Nutritional Biochemical Co.) as nitrogen source (N equivalent 0.5 g per liter). The vitamin supplement was then added to half of each preparation and Petri dishes were poured, inoculated, and incubated as previously described.

Growth was determined as the diameter of the fungus colony after 60 hours for *T. viride* and 156 hours for the other fungi. The data are recorded in Table I. Neither *O. graminis* nor *F. annosus* made satisfactory growth in the absence of the vitamin supplement on any nitrogen source except yeast hydrolyzate.

TABLE I

Growth of *Trichoderma viride*, *Trichocladium asperum*, *Ophiobolus graminis*, and *Fomes annosus* on four nitrogen sources with and without a vitamin supplement

Nitrogen source	Vitamin supplement	Mean diameter of colony (mm)*			
		T. viride	*T. asperum*	*O. graminis*	*F. annosus*
Potassium nitrate	−	58.6	29.6	10.0	7.0†
	+	59.0	30.3	40.6	33.3†
Ammonium sulphate	−	48.3	—	—	4.0
	+	42.3	—	—	20.0
Asparagine	−	58.6	26.0	12.0	12.3
	+	59.3	26.0	58.0	37.3
Yeast hydrolyzate	−	70.6	31.3	57.0	51.0
	+	72.3	32.6	56.3	60.3

T. viride incubated for 60 hours, other fungi for 156 hours.
†Starvation type growth.

This was in contrast to the results with *T. viride* and *T. asperum*, which gave no indication of a vitamin requirement. It may be presumed that the vitamins supplied in the yeast hydrolyzate satisfied the requirements of the two parasites. All four fungi made some growth with KNO_3 in the presence of the vitamin supplement. Growth of *F. annosus*, however, commenced only after an 84-hour lag and consisted of starvation type hyphae, and hence the measurements obtained are not truly comparable. These results are in agreement with those of others who have found nitrate to be an unsatisfactory nitrogen source for *F. annosus* and related species (13, 16). The utilization of nitrate by *O. graminis* in the presence of vitamins was demonstrated by Garrett (9) and subsequently by White (33), but it appears to be less satisfactory than the organic nitrogen sources.

Ammonium sulphate did not support growth of either *T. asperum* or *O. graminis*, and that of *T. viride* and *F. annosus* was less than with KNO_3. Hacskaylo *et al.* (13) found KNO_3 and asparagine to be satisfactory nitrogen sources for *T. viride* and $(NH_4)_2SO_4$ to be less favorable. It is possible that the SO_4^- radical was toxic to *T. asperum* and *O. graminis*, and to a lesser degree to *T. viride* and *F. annosus*, and that NH_4^+ might be utilized when differently combined. White (33) demonstrated that NH_4NO_3 could serve as a nitrogen source for *O. graminis* but it is possible that only the NO_3^- radical was utilized.

Yeast hydrolyzate and *l*-asparagine were good nitrogen sources for all four fungi. Growth of *O. graminis* with *l*-asparagine was equal to that with yeast hydrolyzate, but the other three fungi, and particularly *F. annosus*, grew better with the latter. It would appear from these results that in addition to possessing vitamin requirements, the two parasites showed a greater preference for organic nitrogen than did the saprophytes.

Experiments were carried out to determine which members of the vitamin supplement were essential for growth of *O. graminis* and *F. annosus*. The solid synthetic medium was modified by deletion of each vitamin in turn or by substitution of each singly for the complete mixture as indicated in Table II. Although the development of starvation type hyphae on unfavorable media by *F. annosus* reduced the apparent sensitivity of the experiment for this fungus, the data of Table II clearly indicate that thiamine was essential for growth of both fungi. In addition, biotin was required by *O. graminis*, and a partial requirement for pyridoxine was indicated for *F. annosus*. White (33) obtained similar results with *O. graminis* except that in his experiments limited growth occurred with biotin alone. The thiamine requirement of *F. annosus* has also been reported previously (6, 16, 24). The growth which

TABLE II

The effect of thiamine-HCl, pyridoxine-HCl, Ca-pantothenate, *d*-biotin, *i*-inositol, niacinamide, and *p*-aminobenzoic acid on the growth of *Ophiobolus graminis* and *Fomes annosus*

Vitamins added	Mean diameter of colony (mm)*	
	O. graminis	*F. annosus*
All	74.3	72.5
All but thiamine-HCl	17.3	36.7†
All but pyridoxine-HCl	71.0	59.5
All but Ca-pantothenate	75.5	66.8
All but *d*-biotin	31.3	71.7
All but *i*-inositol	73.5	70.5
All but niacinamide	72.5	72.3
All but *p*-aminobenzoic acid	75.5	67.0
Thiamine-HCl	30.0	63.8
Pyridoxine-HCl	15.5	39.7†
Ca-pantothenate	17.3	35.9†
d-Biotin	16.0	36.5†
i-Inositol	15.0	39.5†
Niacinamide	14.5	35.8†
p-Aminobenzoic acid	14.5	37.0†
Control	19.8	38.5†

*Incubated for 10 days.
†Starvation type growth.

occurred in the absence of the required vitamins was presumably due to carry-over of vitamins with the inoculum or due to impurities in the agar.

Influence of Carbohydrates on Growth

Four groups of carbohydrates were considered in these experiments: hexose and pentose monosaccharides, disaccharides, and polysaccharides. These were substituted in equimolar amounts for D-glucose in the synthetic medium. Each group was tested separately but a glucose control was included in each experiment to provide a basis for comparison both between groups and between fungi. Growth was determined on liquid media as the mean dry weight of mycelium harvested from four or five replicate culture flasks for each carbohydrate.

Hexose Monosaccharides

Growth was compared on four hexoses and the results obtained are indicated in Table III.

All four fungi behaved similarly: growth with D-galactose was poorer than with D-glucose, while that with D-fructose tended to be slightly better. However, the differences in growth with D-glucose, D-fructose, and D-mannose were small. L-Sorbose was not included in these experiments but preliminary tests had indicated that only *T. asperum* was able to make limited growth with this sugar.

TABLE III

Growth of *Trichoderma viride*, *Trichocladium asperum*, *Ophiobolus graminis*, and *Fomes annosus* on a synthetic medium with hexose monosaccharides as carbon sources

Carbon source	Yield dry mycelium (mg)*			
	T. viride†	*T. asperum*‡	*O. graminis*§	*F. annosus*‖
D-Glucose	97.9 ± 4.8	125.6 ± 6.5	141.7 ± 3.8	64.7 ± 6.6
D-Fructose	107.1 ± 4.1	135.7 ± 5.1	145.9 ± 4.2	73.9 ± 7.0
D-Mannose	94.7 ± 2.5	123.6 ± 4.9	141.1 ± 5.3	74.6 ± 3.8
D-Galactose	87.8 ± 3.1	98.1 ± 17.9	104.7 ± 7.8	51.6 ± 7.8

*Mean of five replicates.
†, ‡, §, ‖ 8, 12, 11, 12 days' growth respectively.

Pentose Monosaccharides

The growth obtained with four pentoses and with D-glucose is shown in Table IV. The four species varied considerably in their ability to utilize the

TABLE IV

Growth of *Trichoderma viride*, *Trichocladium asperum*, *Ophiobolus graminis*, and *Fomes annosus* on a synthetic medium with pentose monosaccharides as carbon sources

Carbon source	Yield dry mycelium (mg)*			
	T. viride†	*T. asperum*‡	*O. graminis*§	*F. annosus*‖
L-Arabinose	32.8 ± 3.1	90.4 ± 8.1	110.6 ± 9.0	44.9 ± 9.7
D-Arabinose	28.9 ± 3.5	30.8 ± 9.7	61.7 ± 6.5	37.1 ± 3.6
D-Ribose	76.6 ± 2.1	35.0 ± 7.1	102.1 ± 10.7	43.3 ± 10.5
D-Xylose	76.1 ± 4.5	121.8 ± 8.0	111.9 ± 12.4	60.5 ± 9.9
D-Glucose	85.5 ± 3.7	134.9 ± 5.6	131.1 ± 9.5	70.3 ± 8.5

*Mean of five replicates.
†, ‡, §, ‖ 10, 10, 14, 14 days' growth respectively.

pentoses, none of which supported as much growth as D-glucose. D-Xylose was uniformly the best carbon source of the pentoses and D-arabinose the poorest. L-Arabinose did not support appreciably more growth than D-arabinose in the case of *T. viride* and *F. annosus*. *O. graminis*, however, grew well with L-arabinose, and *T. asperum* made more growth with this than with the D-form. D-Ribose was a poor carbon source for *T. asperum* and *F. annosus* but was satisfactory for *T. viride* and *O. graminis*.

Disaccharides

Five common disaccharides were compared with D-glucose as carbon sources for the four species under study. The results obtained are given in Table V where differences in the utilization of the various sugars are clearly indicated.

TABLE V

Growth of *Trichoderma viride*, *Trichocladium asperum*, *Ophiobolus graminis*, and *Fomes annosus* on a synthetic medium with disaccharides as carbon sources

Carbon source	Yield dry mycelium (mg)*			
	T. viride†	*T. asperum*‡	*O. graminis*§	*F. annosus*‖
Maltose	69.7 ± 12.0	131.4 ± 4.1	94.2 ± 16.2	96.0 ± 8.3
Sucrose	50.8 ± 12.9	132.5 ± 2.7	114.6 ± 2.4	80.2 ± 6.4
Lactose	96.3 ± 18.1	63.2 ± 12.1	136.5 ± 12.4	87.8 ± 2.0
Melibiose	98.5 ± 24.5	46.5 ± 7.9	49.8 ± 14.9	73.3 ± 5.9
Cellobiose	110.7 ± 11.8	135.8 ± 7.9	134.9 ± 8.3	61.3 ± 3.3
D-Glucose	89.1 ± 14.9	129.1 ± 5.1	119.3 ± 9.6	92.1 ± 3.1

*Mean of four replicates.
†, ‡, §, ‖ 10, 10, 10, 15 days' growth respectively.

Growth of *T. viride* and *F. annosus* with maltose was inferior to that with D-glucose, and sucrose was not utilized as readily as D-glucose by these two fungi. Lactose was relatively unavailable to *T. asperum* but was readily utilized by the other species. Only *T. viride* grew as well with melibiose as with D-glucose, and this was a very poor carbon source for *T. asperum* and *O. graminis*. Good growth occurred with cellobiose except in the case of *F. annosus*.

Polysaccharides

Growth of the four species was determined with inulin, starch, and pectin as carbon sources and compared to that with D-glucose. The results are given in Table VI.

TABLE VI

Growth of *Trichoderma viride*, *Trichocladium asperum*, *Ophiobolus graminis*, and *Fomes annosus* on a synthetic medium with polysaccharides as carbon sources

Carbon source	Yield dry mycelium (mg)*			
	T. viride†	*T. asperum*‡	*O. graminis*§	*F. annosus*‖
Inulin	46.0 ± 3.5	126.8 ± 3.1	76.9 ± 1.4	58.6 ± 10.9
Starch	108.0 ± 17.1	125.0 ± 8.5	126.3 ± 5.7	111.8 ± 5.1
Pectin	69.3 ± 3.0	98.4 ± 2.7	100.5 ± 10.7	103.1 ± 12.7
D-Glucose	91.0 ± 4.5	126.6 ± 6.9	143.7 ± 6.7	100.3 ± 4.1

*Mean of four replicates.
†, ‡, §, ‖ 10, 10, 14, 16 days' growth respectively.

Inulin was poorly utilized by all species except *T. asperum* for which it supported growth equal to that with D-glucose. All species grew well with starch, but only *F. annosus* used pectin as readily as D-glucose.

Summary

In order to compare the effect of the sugars used in different experiments on growth of the four fungi, the dry weight values have been converted to percentages of the yields with D-glucose and are given in Table VII. D-Glucose, D-fructose, and D-mannose were uniformly the best carbon sources for all four species. Growth with D-glucose was exceeded by that with some sugars, but differences were not significant.

TABLE VII

Growth of *Trichoderma viride, Trichocladium asperum, Ophiobolus graminis,* and *Fomes annosus* on carbohydrates, estimated as a percentage of that on D-glucose

Carbon sources	*T. viride*	*T. asperum*	*O. graminis*	*F. annosus*
D-Glucose	100.0	100.0	100.0	100.0
D-Fructose	109.4	108.0	103.0	114.3
D-Mannose	96.7	98.4	99.6	115.3
D-Galactose	89.6	78.1	73.9	79.7
L-Arabinose	38.4	67.0	84.4	63.9
D-Arabinose	33.8	22.9	47.1	52.8
D-Ribose	89.6	25.9	77.9	61.6
D-Xylose	89.0	90.2	85.4	86.1
Maltose	78.2	101.8	78.9	104.2
Sucrose	57.0	102.6	96.0	87.1
Lactose	108.1	48.0	114.4	95.4
Melibiose	110.6	26.0	41.7	80.6
Cellobiose	124.2	105.2	113.1	66.5
Inulin	50.5	100.2	53.5	58.4
Starch	118.6	98.8	87.9	111.5
Pectin	76.1	77.8	69.9	102.8

The pentoses as a group were poorly utilized in comparison with the other three groups of carbohydrates, although D-xylose supported good growth of all four fungi. D-Arabinose was the only carbohydrate tested with which growth of all four fungi was poor.

Utilization of the di- and poly-saccharides was quite varied. The results indicate, when considered together with those for the hexoses, that the fungi differed considerably in their ability to hydrolyze these compounds. It is possible that the formation of adaptive enzymes was required in certain cases, and in these experiments a distinction could not be made between poor utilization of a compound and a lag in the onset of enzymic breakdown. However, both possibilities are likely to place a fungus at a disadvantage in the competitive utilization of such compounds.

Discussion

The results of the experiments reported here tend to emphasize, primarily, the individuality of the four fungi studied. An over-all distinction between the behavior of the saprophytic and parasitic species would be difficult to make.

However, the two parasites were similar and distinct from the saprophytes in that they required vitamins for growth and showed a preference for organic nitrogen. Cook and Lochhead (4) have shown that bacteria capable of synthesizing growth substances are much more prevalent in the rhizosphere and at the root surface than in control soil, and suggest that they may provide a major source of vitamins for fungi active in the root zone. Amino acid requiring bacteria have been found in greater numbers in rhizosphere than in non-rhizosphere soil (19) and this has been explained in the light of reports of liberation of amino acids from plant roots (12, 17). These findings suggest that from the point of view of vitamin and nitrogen nutrition in the soil, optimum conditions for the growth of *O. graminis* and *F. annosus* are likely to be found in close proximity to plant roots, and this is consistent with the ecological status of these fungi. In soil remote from the root surface both fungi might be at a considerable disadvantage, especially in comparison with fungi such as *T. viride* and *T. asperum*, which are satisfied with inorganic nitrogen and are independent of an external supply of vitamins.

The temperature responses of the four fungi were quite varied and suggest that temperature may be a critical factor in the interrelationship of fungi in their natural soil environments.

T. viride grew very rapidly at temperatures close to its optimum (25–30° C) but its growth rate was markedly reduced at lower temperatures. It may be expected that in the soil *T. viride* would have a considerable advantage in growth rate over slower growing fungi such as *O. graminis* in the 20–30° C range, but at lower temperatures this advantage would be reduced and at 10° C and below the situation might be reversed. Henry (14) demonstrated that in unsterilized soil infection of wheat seedlings by *O. graminis* was severe at 13° C but almost absent at 27° C, presumably due to a differential effect of temperature on the antagonistic soil microflora. The temperature responses reported for *T. viride* and *O. graminis* by Ludwig (21) and in the present investigation may explain these results, particularly in view of the evidence obtained by Ludwig and Henry (22), which indicated that *T. viride* was one of the main antagonists of *O. graminis* in recontaminated sterilized soil.

The growth rate of *T. asperum* varied with temperature in a manner similar to that of *O. graminis* but was slower over the entire range. This fungus, which was readily isolated from natural soil, lacks the property of high growth rate suggested by Garrett (11) to be a frequent characteristic of successful saprophytes.

Growth of *F. annosus* did not occur at 15° C during the incubation period used. In its optimum temperature range, however, it grew more rapidly than *T. asperum*, after a lag, and at 30° C continued growth after that of *O. graminis* and *T. asperum* had been arrested.

In the investigation of the effect of pH on growth, the two parasites were shown to be much less tolerant of acidity than were the two saprophytes. Growth of both *O. graminis* and *F. annosus* fell sharply at pH values below 5.0 whereas that of *T. viride* was only slightly less at pH 3.1 than at neutrality and *T. asperum* grew particularly well at pH 4.0. The intolerance of acidity by *O. graminis* is consistent with the observation that 'take all' is more prevalent under alkaline than acid soil conditions, a correlation first noted by Kirby

(18). Garrett (7), however, suggested that the difference in activity of *O. graminis* in acid and alkaline soils was due to the inhibitory effect of an accumulation of carbon dioxide around plant roots in the former, while alkaline soils were thought to reduce the carbon dioxide concentration by combining it chemically. This view was based chiefly upon the observations that increased aeration overcame the depressing effect of acidity on *O. graminis* (8); carbon dioxide in high concentrations was inhibitory to growth of the fungus on potato-dextrose agar (7); and a variation in the pH of sterilized soil between 6.5 and 7.5 was without effect on growth of the fungus (7). However, with the isolate used in the present investigation no appreciable difference in growth would be expected over the pH range 6.5–7.5, and the other evidence does not necessarily exclude the possibility that the reduced activity of *O. graminis* in acid soils is due, at least partly, to its intolerance of low pH. In addition, acidity, in reducing the growth of *O. graminis*, may place it at a considerable disadvantage in natural soil in competition with more acid tolerant, and possibly antagonistic, members of the microflora, such as *T. viride*. Rishbeth (25, 26, 27) has in fact used an explanation similar to this to account for the greater severity of *F. annosus* on pine roots in alkaline soils than in more acid conditions. *T. viride* was very prevalent on root surfaces in acid soils and was shown experimentally to inhibit and overgrow *F. annosus*. On the basis of the acid pH ranges determined for these two fungi in the present investigation *T. viride* would most certainly have a distinct advantage over *F. annosus* under acid conditions.

There appear to be no definite trends in carbohydrate utilization characteristic of any of the four fungi investigated here. However, the individual differences in growth which were found with many of the carbohydrates suggest that in soil microhabitats, availability of specific carbon compounds might have a selective effect on members of the microflora.

The isolation plate methods (31) used for the isolation of fungi from soil or from infected plant roots depend for their success on the ability of specific fungi to grow onto the surface of agar media in competition with other microorganisms in the soil particles or tissue segments. For this reason Garrett (11) has suggested that the isolation plate may be used as a model to illustrate competitive saprophytic ability among fungi. However, the common use of selective media for the isolation of specific fungi indicates that their relative competitive saprophytic ability can be altered by the conditions employed, and this is supported by the results obtained here. The differences in response of the four fungi to nutritional and physical factors suggest that no single combination would suit all equally well, although selective conditions might be found for each. The nature of the substrate and other environmental conditions should be taken into consideration, therefore, in the interpretation of investigations of microbial competition. Failure to do so may lead to erroneous conclusions. Rao (23), for example, used isolation plates to determine the competitive saprophytic ability of several fungi, including *O. graminis* and *F. annosus*. It was found that neither of these fungi grew onto isolation plates from mixtures of inoculum and unsterilized soil even when the inoculum constituted 98% of the mixture. According to this test both fungi were completely lacking in competitive saprophytic ability. However, the pH of

the medium used was 4.0 and contained $NaNO_3$ as nitrogen source, and the results obtained may well be explained by the relative unsuitability of this medium for growth of these two fungi.

Although fungi are unlikely to grow in mixed populations under conditions which do not support their growth in pure culture, it is not suggested that optimum pure culture conditions are necessarily those which provide greatest competitive advantage in natural soil. The ascendancy of a particular species in a competing population may be encouraged most by the provision of conditions relatively less favorable to competitors. The differential effect of cool temperatures in favor of *O. graminis* has already been discussed, and may provide an example of this type of situation. The experiments of Butler (2) and Lucas (20) indicate that *O. graminis* is a poor competitor on dead wheat straw in soil at room temperature. It would be of considerable interest, however, to determine if lower temperatures or alteration of other environmental conditions could enhance the competitive saprophytic ability of this fungus in experiments of this kind.

The differences found in the response of the four fungi studied here to various cultural conditions suggest that they would differ frequently in their reaction to natural environmental conditions. Obviously, the combinations of physical and nutritional factors used are only a fraction of those theoretically possible and likely to occur in nature. In the soil the existence of any single combination of environmental factors of this type will probably be limited both in space and time according to the presence and proximity of mineral and organic nutrients and to fluctuations in temperature and precipitation. As each combination blends into another a corresponding change in the relative activity of members of the microflora is to be expected. The full complexity of the soil environment is only realized, however, when consideration is given to the numerous biological factors which greatly multiply the almost limitless variations of the microhabitat already indicated. The determination of physical and nutritional factors governing the growth of soil-inhabiting and root-infecting fungi may provide a basis from which further analysis of their ecology can be made.

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CYTOLOGICAL STUDIES OF THE DEVELOPMENT OF METAPHASE I IN HYBRIDS BETWEEN TRITICUM TIMOPHEEVI ZHUK. AND T. DURUM DESF.[1]

E. B. WAGENAAR[2]

Abstract

In two hybrids between *Triticum timopheevi* Zhuk. and *T. durum* Desf., which have irregular meioses, metaphase I was subdivided into four developmental stages, early, medium, late, and very late. This subdivision was based on the presence in the anthers of other stages that occurred together with metaphase I. It was then discovered that in metaphase I cell populations there was a progressive increase of univalents from the early and medium stages to the very late stage. This phenomenon can be explained on the assumption that metaphase I is of shorter duration in the less irregular cells which pass into anaphase I earlier than the more irregular cells. As a consequence of this developmental phenomenon at metaphase I, the anaphase I and telophase I cells in the late anthers contained fewer lagging chromosomes than the anaphase I and telophase I cells in the very late anthers. Despite these numerical differences, the degrees of lagging were remarkably similar in both stages; approximately 70% of these univalents lagged at late and very late stages in both hybrids.

During metaphase I many univalents of the irregular cells moved towards the equatorial plate, became oriented, and lagged at anaphase I and telophase I. It was found that the univalents of the least irregular cells accumulated somewhat faster at the plates than those of the more irregular cells.

Considering the relationships between all of the available data, the hypothesis is advanced that when a certain number of univalents have accumulated at the equatorial plate a state of equilibrium is established and anaphase I is initiated. On the basis of this hypothesis an explanation of the trends observed at metaphase I is given.

Introduction

In hybrids with irregular meioses it has often been found that the average numbers of bivalents and other chromosome associations vary significantly. These variations may be observed not only in anthers obtained from different spikes of the same hybrid, but they may also occur in anthers of the same spike (1, 9, 12). The phenomenon is usually attributed to differences in environmental conditions to which these anthers or spikelets were exposed.

Similar differences in metaphase I averages occurred during a cytological study on irregular meioses of some hybrids between *Triticum timopheevi* Zhuk. ($2n = 28$) and *T. durum* Desf. ($2n = 28$). A detailed investigation revealed that these differences were not due to environment but to the chromosomes whose behavior had a direct effect on the duration of metaphase I and on the number of lagging chromosomes at anaphase I and telophase I.

The present paper includes the data obtained from observations of metaphase I, anaphase I, and telophase I in two hybrids between *T. timopheevi*

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and *T. durum*. On the basis of the analysis of the data a hypothesis is given to account for the developmental phenomenon that was found to occur at metaphase I.

Materials and Methods

In this study two hybrids were cytologically investigated, namely, *T. timopheevi* × *T. durum* var. *leucomelan* and *T. durum* var. *obscurum* × *T. timopheevi*. Hereafter these hybrids will be referred to as hybrid I and hybrid II, respectively. Two spikes of each hybrid were analyzed. The F_1 plants of each hybrid were grown in two separate clumps in the field in 1958, so that it is not known whether the spikes of each hybrid were obtained from one or two plants. The spikes of hybrid I were collected on the same day, while those of hybrid II were collected on 2 different days. Since the meiosis of hybrid I was most intensively investigated the data of this hybrid will be discussed in full detail. Hybrid II was used as a check to confirm the trends observed in hybrid I. Most of the data of this hybrid will, therefore, be given in more or less summarized form.

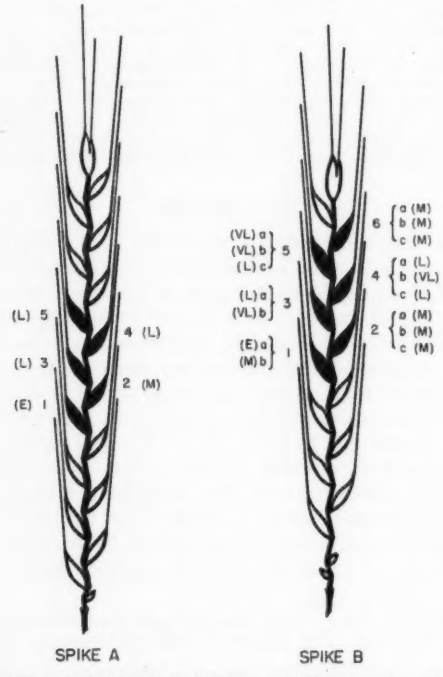


FIG. 1. Young spikes of hybrid I. Spikelets from which florets were taken for analysis are indicated in black. For spike A one slide was made from the three anthers of each of the numbered florets, while for spike B a slide was made from each anther identified by number and letter. The stage of development of each floret or anther analyzed is indicated.

For the analysis of hybrid I, five slides were prepared from spike A, each slide containing the squashed contents of the three anthers of a single floret, and 16 slides were prepared from spike B, each comprising the contents of a single anther. The positions of these florets and anthers are shown in Fig. 1.

The slides were then classified as follows:

early (E): slides with cells at diakinesis and metaphase I;
medium (M): slides with cells at metaphase I only;
late (L): slides with cells at metaphase I, anaphase I, and telophase I;
very late (VL): slides with cells at metaphase I, anaphase I, telophase I, and interphase.

With the slides classified this way, it was possible to study the events that took place at metaphase I in anthers that were at four different stages of development.

The two spikes of hybrid II were analyzed in the same manner. A total of nine slides, each containing the squashed contents of one anther, were studied.

It should be emphasized here that the cytological data were studied on a population basis. This approach will be particularly clear in the sections that deal with the statistical analysis of some of the data.

Results

1. The Developmental Pattern of Metaphase I

The cytological data collected from metaphase I cells of hybrid I are presented in Table I. Each column shows the sample of metaphase cells belonging to one of the four developmental stages, which are indicated by the letters E, M, L, and VL in the column heading. Slides at stage E yielded few metaphase cells since at early metaphase I the chromosomes were often so tightly grouped that reliable analyses were difficult and often impossible. The population of the metaphase cells observed in VL anthers was also relatively small. From 21 slides a total of 1935 cells was analyzed which had an average of 6.66 univalents, 9.04 bivalents, 1.06 trivalents, and 0.03 quadrivalents.

Table I shows that cells with one trivalent occurred most frequently (70.3% in spike A and 66.6% in spike B). Cells with two trivalents (10.6% in spike A and 18.7% in spike B), and cells without trivalents (14.4% in spike A and 9.5% in spike B) occurred in a relatively low frequency (Figs. 3, 4, 5, and 6). The category, classified as "other cells", contained cell classes which occurred in very low frequencies, as for example, cells with three trivalents, or with one quadrivalent and one trivalent, etc.

Table I further shows that the three populations of metaphase cells were heterogeneous, containing several subpopulations of which the three principal ones were cells with no trivalents, cells with one trivalent, and cells with two. The modal class for each of these subpopulations is indicated by italics. In most modal classes of the L and VL stages there were more univalents than in the E and M stages. This tendency was especially clear in the large subpopulation having one trivalent.

Figure 2 is a graphical representation of the data shown in Table I. For this figure the cells were reclassified according to the number of univalents. The four bargraphs represent the frequency of metaphase I categories in each of

TABLE I

Frequencies in percentages of cell categories at metaphase I of spikes A and B of hybrid I, grouped into four developmental stages E, M, L, and VL

Chromosome associations per cell category			Developmental stages of spike A				Developmental stages of spike B				
III	II	I	Early	Medium	Late	Total	Early	Medium	Late	Very late	Total
	14		—	—	0.2	—	—	—	—	—	—
	13	2	—	—	0.2	—	—	0.4	0.5	—	0.4
	12	4	—	3.9	1.8	2.0	—	0.9	1.2	—	0.8
	11	6	6.4	2.6	3.4	3.4	8.3	*2.6*	1.7	1.4	2.2
	10	8	6.4	2.6	*3.8*	*3.8*	—	2.4	*2.2*	2.8	*2.4*
	9	10	—	—	2.5	2.0	—	1.5	1.7	2.5	1.7
	8	12	3.2	—	2.0	1.8	—	1.3	0.5	*4.9*	1.8
	7	14	—	—	1.3	1.1	—	—	0.2	0.7	0.2
1	12	1	3.2	6.5	0.9	1.8	—	4.0	2.0	—	2.5
1	11	3	9.7	10.4	5.2	6.1	16.7	10.7	5.0	1.4	7.2
1	10	5	*38.7*	19.5	16.1	17.8	*50.0*	*20.0*	18.6	8.5	17.5
1	9	7	12.9	*27.3*	*25.1*	*24.7*	16.7	18.4	*21.8*	16.2	*18.9*
1	8	9	3.2	9.1	14.5	13.2	8.3	9.6	12.4	*25.0*	13.6
1	7	11	3.2	1.3	5.8	5.0	—	3.4	4.5	10.9	5.2
1	6	13	3.2	—	1.3	1.3	—	0.9	1.5	2.8	1.5
1	5	15	—	1.3	0.2	0.4	—	0.1	0.5	0.4	0.3
2	10	2	—	1.3	0.9	0.9	—	2.6	1.5	1.1	2.0
2	9	4	—	3.9	2.5	2.5	—	5.4	5.0	3.9	4.9
2	8	6	3.2	2.6	*4.3*	*4.0*	—	*6.8*	*8.2*	*7.0*	*7.2*
2	7	8	3.2	2.6	2.2	2.3	—	2.4	3.5	4.2	3.0
2	6	10	—	—	0.9	0.7	—	0.9	1.0	2.1	1.2
2	5	12	—	—	0.2	0.2	—	0.1	0.5	—	0.2
Other cells			3.2	5.2	407	4.7	—	5.6	6.2	4.2	5.4
No. of cells analyzed			31	77	447	555	12	680	404	284	1380

the four developmental stages. It is noteworthy that the graphs are strongly serrated. The high points occur in classes with odd numbers and the low points in those with even numbers of univalents. The high peaks in the curves are caused by the frequent occurrence of cells having one trivalent, and, therefore, an odd number of univalents. The figure further shows that the frequency curves of E and M are very much alike, although the small cell sample of E (43 cells) may have caused the relatively high number of cells observed in the modal class represented by five univalents.

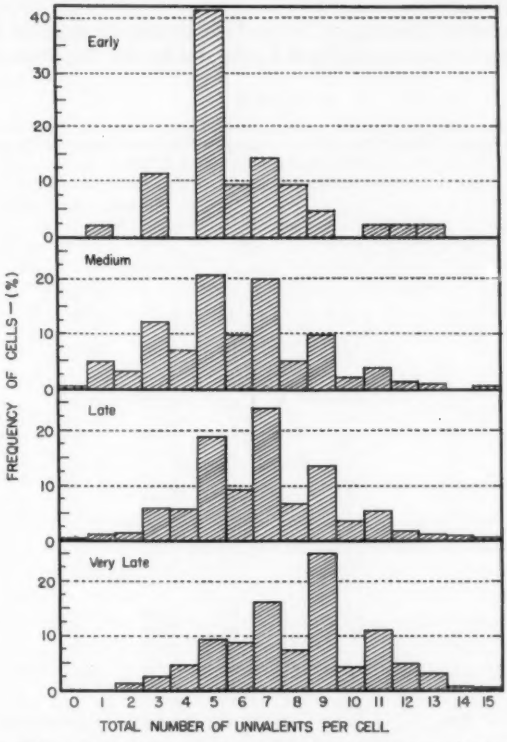


FIG. 2. Bargraphs showing frequencies of metaphase I cell categories in early, medium, late, and very late stages of metaphase I of hybrid I.

When the graphs representing metaphase I populations from M, L, and VL anthers are compared it is obvious that in L and VL anthers the cells had more univalents; the averages for M, L, and VL anthers were 5.88, 6.86, and 8.17 univalents, respectively. There was thus a clear trend for the metaphase cell population to become more irregular in the L and the VL stages.

Table II shows the average values for the various chromosome associations in metaphase cells observed in each of the slides that were classified according

to the stage of development of the floret or anther. From this it can be seen that there was more homogeneity within than between the four developmental stages. In the VL stages where the number of univalents per metaphase I cell increased by approximately two, the increase occurred chiefly at the expense of bivalents since the average formation of trivalents was practically unchanged. Since there was some fluctuation in the average numbers of bivalents and trivalents that were formed, the total amount of pairing or non-pairing at metaphase I can, therefore, be best expressed in terms of the numbers of univalents that were present.

When considering the data in Table II it should be kept in mind that the changes that occurred in metaphase I cells during development of the anthers

TABLE II

Average numbers of chromosome associations and univalents of metaphase I cells of hybrid I for each slide classified according to the developmental stages early, medium, late, and very late

Spike No.	Slide No.	No. cells	Av. no. chromosome associations				
			I	II	III	IV	V
			Early				
A	1	31	6.22	9.45	0.90	—	0.03
B	1[a]	12	5.42	9.92	0.92	—	—
Average		43	6.00	9.58	0.91	—	0.02
			Medium				
A	2	77	5.88	9.47	1.03	0.03	—
B	1	79	5.71	9.73	0.92	0.01	—
B	2[a]	90	5.66	9.36	1.10	0.07	0.01
B	2[b]	144	5.70	9.22	1.20	0.05	0.01
B	2[c]	153	6.23	9.14	1.16	0.01	—
B	6[a]	52	5.71	9.44	1.13	—	—
B	6[b]	79	5.94	9.22	1.15	0.04	—
B	6[c]	83	6.27	9.20	1.07	0.01	—
Average		757	5.88	9.28	1.10	0.03	—
			Late				
A	3	110	6.96	9.00	0.96	0.04	—
A	4	163	7.06	8.93	0.94	0.07	—
A	5	174	7.20	8.94	0.94	0.02	—
B	3[a]	120	6.48	9.03	1.13	0.03	—
B	4[a]	122	6.31	9.05	1.13	0.06	—
B	4[c]	100	7.33	8.56	1.13	0.04	—
B	5[c]	62	6.27	9.05	1.21	—	—
Average		851	6.86	9.03	1.04	0.04	—
			Very late				
B	3[b]	41	7.59	8.51	1.10	0.02	—
B	4[b]	95	8.43	8.23	1.02	0.01	—
B	5[a]	66	8.20	8.11	1.08	0.09	—
B	5[b]	82	8.15	8.35	1.05	—	—
Average		284	8.17	8.28	1.05	0.03	—
Average spike I		555	6.87	9.05	0.96	0.04	—
Average spike II		1380	6.57	8.97	1.11	0.03	—
Average spikes I + II		1935	6.66	9.04	1.06	0.03	—

from E to VL were continuous. Because of this the arbitrary classification of an individual anther with respect to its stage of development could not always be precise, particularly if it was midway between two stages. This introduced a certain degree of fluctuation in average bivalent and univalent frequencies, to which must also be added the variation due to sampling error. In spite of these factors the variations within a developmental group were small in comparison to the differences that existed between the stages M, L, and VL.

The populations of metaphase cells in Table I can be divided into subpopulations according to the numbers of trivalents that were present in the cells. In Table III these subpopulations are presented for each of the three developmental stages M, L, and VL. Stage E is deleted on account of its small total population.

TABLE III

Cell frequencies and averages of numbers of univalents for each of the four subpopulations of metaphase I cells of hybrid I in the three developmental stages M, L, and VL

Subpopulations*	Medium			Late			Very late		
	No. cells	%	Av. no. univalents	No. cells	%	Av. no. univalents	No. cells	%	Av. no. univalents
No trivalents	77	10.2	7.19	116	13.6	7.84	39	13.7	9.74
One trivalent	528	69.9	5.95	594	69.8	6.91	190	66.9	8.36
Two trivalents	137	18.1	5.21	133	15.6	5.91	53	18.7	6.30
Three trivalents†	13	1.7	—	8	0.9	—	2	0.7	—
Total	755‡			851			284		

*A quadrivalent was considered as two bivalents.
†Since cell frequencies were very small, no averages were calculated.
‡Two cells with a pentavalent were excluded.

Each subpopulation showed the same trend as was found in the population as a whole. There was an increase in the average number of univalents as the development of the anther proceeded. However, the averages for the different stages, and the degree of increase between the stages, were different for each of the subpopulations. For example, metaphase cells with two trivalents showed an increase of only one univalent from M to VL stages, while those with one trivalent (the largest subpopulation) showed an increase of 2.4 univalents.

Table III also shows that in all the three stages M, L, and VL, the average number of univalents was highest for the subpopulation of cells that had no trivalents. This is not unexpected, since for each cell in which a bivalent was formed instead of a trivalent, there was a net gain of one univalent. It was stated earlier that the increase in the average number of univalents that was observed in the cell populations of L and VL anthers occurred at the expense of the bivalents. The validity of this can readily be seen since the frequencies of the trivalents are fairly constant in all four developmental stages. However, comparing the subpopulations within each of the developmental stages, it is evident that the greater average number of univalents of the subpopulation without a trivalent resulted chiefly from failure of trivalent formation.

On the basis of the averages obtained for metaphase I cells (Table II) the following ranges, expressed in univalents per cell, may be established for the developmental stages of the anthers of this hybrid:

early	5.6 to 6.3 univalents,
medium	5.6 to 6.3 univalents,
late	6.4 to 7.3 univalents,
very late	7.4 to 8.5 univalents.

These limits are approximate rather than precise. Note that the average value of 6.66 univalents for all cells analyzed (Table II) lies within the range of the L stage.

The shift towards greater irregularity at late metaphase I observed in hybrid I was also found to occur in the metaphase I cell populations of hybrid II. As shown by the data in Table IV this hybrid exhibited less pairing and, consequently, showed more univalents at metaphase I in all stages of anther development than in hybrid I (Table I). The average number of chromosome associations for each of three stages of anther development in the second hybrid are given in Table V.

The data in Tables IV and V show an increase in univalent frequencies in metaphase cells of anthers at later developmental stages similar to that observed in the previous hybrid (Table II). The cells that remained longest at metaphase I, and proceeded latest into anaphase I, had fewer chromosome associations and, consequently, more univalents. The VL stage had an average of 2.3 univalents more than the M stage (Table V).

TABLE IV

Frequencies expressed in percentage of metaphase I cells of hybrid II arranged according to the number of univalents and grouped in stages M, L, and VL*

No. univalents	Medium	Late	Very late	Total
0	—	—	—	—
1	1.2	—	—	—
2	0.8	—	—	0.5
3	5.2	0.4	0.9	3.5
4	4.3	2.1	—	3.3
5	11.2	6.6	4.6	9.3
6	5.2	4.1	3.7	4.8
7	15.7	10.8	10.2	13.8
8	6.5	8.3	2.8	6.5
9	*19.9*	*20.7*	19.4	*20.0*
10	5.8	5.4	2.8	5.4
11	8.2	16.6	*23.1*	12.0
12	5.8	7.5	6.5	6.3
13	4.3	7.1	9.3	5.6
14	2.8	3.7	4.6	3.3
15	1.0	3.3	5.6	2.1
16	1.3	2.5	1.9	1.7
17	0.5	0.4	1.9	0.6
18	—	—	0.9	0.1
19	0.2	—	1.9	0.3
20	—	0.4	—	0.1
No. cells analyzed	599	241	108	940

*Anthers in E stage were not found.

TABLE V

Average numbers of chromosome associations and univalents in cells at metaphase I in hybrid II

Developmental stage	No. cells	Chromosome associations			
		I	II	III	IV
Medium	599	8.26	8.46	0.92	0.01
Late	241	9.71	7.81	0.87	0.01
Very late	108	10.58	7.22	0.99	—
Average	948	8.90	8.16	0.92	0.01

The use of the changing proportions of metaphase I, anaphase I, telophase I, and interphase cells as a criterion of anther development in order to arrange them into a developmental sequence depends for its validity on whether or not the prophase cells enter metaphase I more or less synchronously. Anthers at the E stage, containing cells at both diakinesis and metaphase, were relatively uncommon in these studies and were found only rarely in other similar hybrids. It was also noticed that the transition occurred more or less per group of cells, i.e. groups of cells were all at diakinesis or all at metaphase I. Groups of cells partly at diakinesis and partly at metaphase I were rarely encountered. This transitional behavior of the cells is of a different kind than that observed between metaphase I and anaphase I, where anaphase cells were scattered among the metaphase cells. These observations indicate that the transition from diakinesis to metaphase I was of brief duration and that it occurred more or less synchronously throughout the anther. If this were not the case, it would be unlikely that a developmental sequence based on the occurrence of metaphase I, anaphase I, telophase I, and interphase cells would yield the definite and continuous trends that have been shown in the data presented earlier.

Hollingshead (8) also found in *T. aestivum* (Marquis) that there was an increase in the percentage of metaphase cells containing univalents as the development of the anthers proceeded. She distinguished five developmental stages, viz., early, early-medium, medium, medium-late, and late, and though she did not clearly indicate how the sequence was determined, she mentioned that anthers classified as late contained cells at interphase. In her study the percentage of cells containing univalents increased from 3.2% in early anthers to 15.2% in late anthers. Her observations also showed that in the late stage the metaphase cells were not localized but scattered at random throughout the anther. She made the same observation, therefore, as the present writer in that there was no relation between the number of univalents in a cell and its location within the anther.

The results obtained by Hollingshead on *T. aestivum* and those obtained in this study on the two hybrids involving *T. timopheevi* both support the interpretation that the transition within an anther from metaphase I to anaphase I is a gradual process in which cells with few univalents proceed into anaphase I earliest, while the most irregular cells, containing the most univalents, pass into anaphase latest. It seems, therefore, that metaphase cells which most closely approach the normal state (all bivalents) are more efficient in their development than cells having many univalents.

Figure 3 presents a diagrammatic picture of the meiotic stages from metaphase I to interphase, inclusive, in anthers of *T. timopheevi* hybrids. The width of the bars, each representing a cell category, is in proportion to the actual frequency found in the M stage in anthers of hybrid I, except for the cell categories with no and with 13 univalents. The latter bars are about twice the actual width, so that they can be reproduced in the graph. The diagram is also inaccurately representative in other respects; for example, the duration of metaphase I is drawn too short in comparison to those of the anaphase I and telophase I, and the progressive increase in duration of metaphase I from cell categories with the lower to the higher numbers of univalents is arbitrarily taken. Nevertheless, it demonstrates clearly how the meiotic stages develop in relation to each other. In the figure the developmental stage of an anther can be represented by a vertical line. When such a line lies within the limits of M, not much difference in the average chromosome associations will occur.

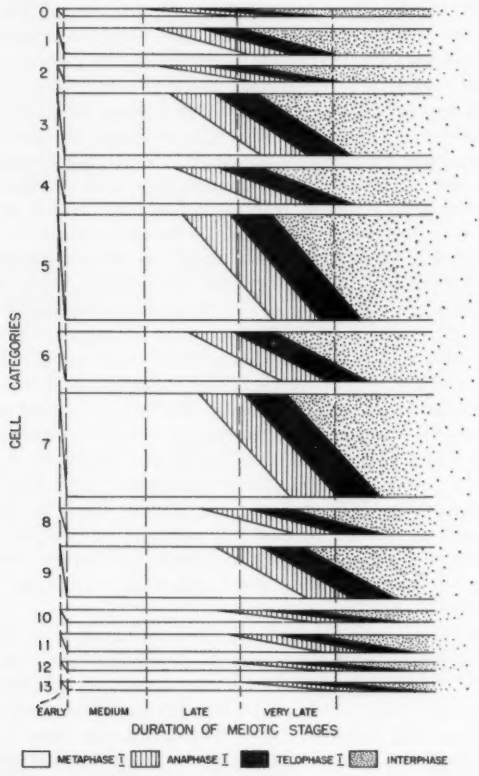
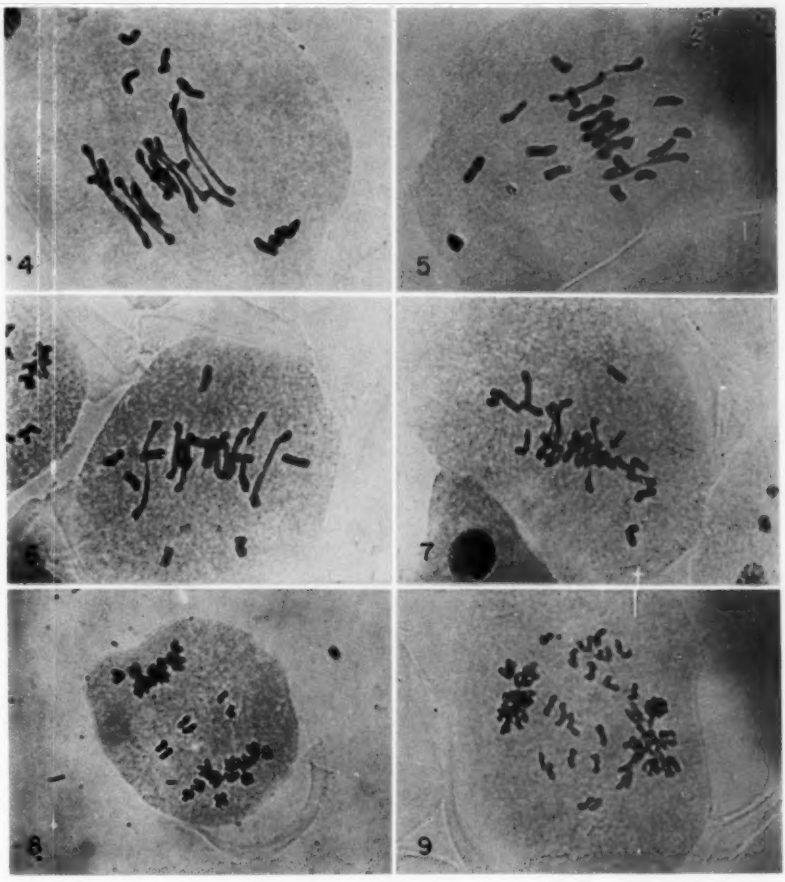


FIG. 3. Diagrammatic representation of the meiotic stages metaphase I, anaphase I, telophase I, and interphase in anthers of *T. timopheevi* × *T. durum* hybrids.



FIGS. 4–9. Pollen mother cells of the hybrid *T. durum* var. *obscurum* × *T. timopheevi* (hybrid II). Magnification approximately 600×.

FIG. 4. Metaphase I in M stage: 9 bivalents, 1 trivalent, and 7 univalents distributed in two groups near poles.

FIG. 5. Metaphase I in L stage: 6 bivalents, 1 trivalent, and 13 univalents. Some univalents moving towards equator. Three univalents located on equatorial plate.

FIG. 6. Metaphase I in VL stage: 8 bivalents, 1 trivalent, and 9 univalents. Five univalents located on the equatorial plate.

FIG. 7. Metaphase I in VL stage: 8 bivalents, and 12 univalents, of which 9 are located on the equatorial plate. Arrow points to two overlapping univalents.

FIG. 8. Anaphase I in L stage: three lagging chromosomes at equatorial region.

FIG. 9. Telophase I in VL stage: nine dividing laggards in center of cell.

But when the line is moved into the region of L, the cell categories with few univalents are partly at anaphase I and telophase I, while the categories with many univalents are still at metaphase I. Consequently, the frequency of the latter metaphase cell categories will increase proportionately. This trend is still more pronounced when the vertical line is moved into the VL region.

From the data presented in this section it is clear that only the chromosome associations found at metaphase I in the M stage can be considered as being representative for the hybrid concerned. For hybrids in which a shift occurs to greater univalent frequencies during the development of metaphase I, the stages L and VL should be disregarded as being unreliable.

2. The Behavior of the Univalents of Metaphase I Cells

Several cytologists have reported that univalents move to and accumulate at the equatorial plate during metaphase I (10, 13, 15, 16). A quantitative study of this phenomenon, however, has never been made. Therefore, in the present study the accumulation of univalents at the equator was recorded for the four developmental stages, E, M, L, and VL. The recording itself was often difficult, for strict rules concerning the positions of the univalents could not always be applied. In general, a chromosome located at the equator within a region having the width of a closed or a small open bivalent was considered to be at the plate (Figs. 4, 5, 6, and 7). No record was made of cells in which no equatorial plate was apparent.

In Fig. 10 and Table VI, both of which show the frequencies of univalents at the metaphase plate of hybrid I for the four developmental stages, the accumulation of univalents at the equatorial plate during metaphase I can be seen. The frequencies of univalents at the plate increased from 20% to approximately 50% (straight line of 45° in Fig. 10), although at the VL stage the frequencies tended toward values higher than 50%. At the L stage the values

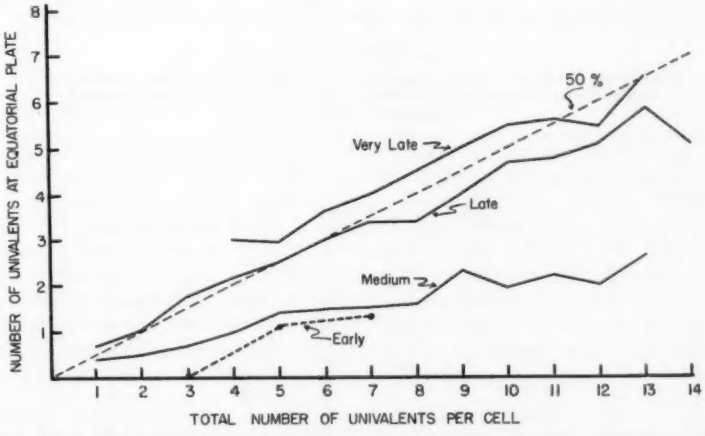


FIG. 10. Accumulation of univalents at the equatorial plate in early, medium, late, and very late stages of metaphase I in hybrid I.

decreased from 67% for cells with one univalent to 56% for cells with four univalents, whereas for cells with five to seven univalents it was approximately 50%. The values of these latter cell categories increased to 55 to 60% at the VL stage. Figure 10 shows that the values of the VL stage generally fell above the straight line which represents an accumulation of 50%.

The accumulation of the univalents at the equator in hybrid I is shown in a different way in Table VII. Here the cells were not classified according to the total number of univalents present in the cells, but according to the number of univalents present at the equatorial plate. In E and M, cells with one univalent at the equator occurred most frequently. In VL the mode was in cells with four univalents at the plate. These figures show clearly the continuous accumulation of the univalents during the development of metaphase I. The trends as shown in Table VII are exaggerated, however, since a large proportion of the metaphase cells with low numbers of univalents were already at anaphase I or telophase I in the L and VL stages.

When the chances of orientation at the equatorial plate *per univalent* are considered (Fig. 11), it appears that the univalents of cell categories with lower total numbers of univalents had somewhat greater chances of moving

TABLE VI

Average number of univalents accumulated at equator of metaphase I cells in the four stages, E, M, L, and VL of hybrids I and II

	Hybrid	Early	Medium	Late	Very late
Average number of univalents per cell	I	6.0	5.9	6.9	8.2
	II	—	8.3	9.6	10.6
Average number of univalents at plate	I	1.3	1.4	3.2	4.4
	II	—	1.1	3.6	4.8
Percentage of univalents at plate	I	21.7	23.7	46.4	53.7
	II	—	13.3	37.4	45.3

TABLE VII

Metaphase I cells of hybrid I classified according to the number of univalents observed at the metaphase plate in each of the four developmental stages E, M, L, and VL

No. univalents at metaphase I plate	E		M		L		VL		Total	
	No. cells	%	No. cells	%	No. cells	%	No. cells	%	No. cells	%
0	11	26.8	197	26.4	32	3.8	3	1.1	243	12.7
1	*16*	*39.0*	*243*	*32.5*	104	12.3	12	4.2	375	19.5
2	7	17.1	184	24.6	162	19.1	23	8.1	*376*	*19.6*
3	5	12.2	79	10.6	*199*	*23.5*	43	15.2	326	17.0
4	2	4.9	34	4.6	175	20.7	*64*	*22.6*	275	14.3
5	—	—	5	0.7	92	10.9	62	21.9	159	8.3
6	—	—	5	0.7	46	5.4	44	15.5	95	5.0
7	—	—	—	—	28	3.3	23	8.1	51	2.7
8	—	—	—	—	8	0.9	5	1.8	13	0.7
9	—	—	—	—	—	—	3	1.1	3	0.2
10	—	—	—	—	1	0.1	1	0.4	2	0.1
Total	41		747		847		283		1918	

towards the equator in the M and L stages than those of cell categories with higher numbers (note concave curves in Fig. 11). Although the VL population contained only cells with four or more univalents, it showed the same trend as observed in the M and L stages. At VL the cell categories with four to nine univalents per cell had the highest chance of accumulating univalents at the equatorial plate, equalling the values obtained for the cell categories with one to four univalents at L.

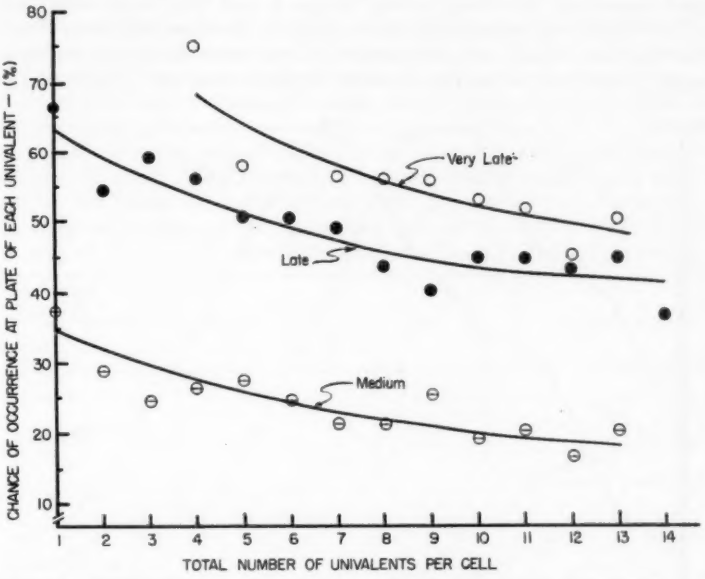


FIG. 11. Chance of occurrence at plate of each univalent of the cell categories at metaphase I for the medium, late, and very late stages of hybrid I.

The behavior of the univalents in hybrid I, as described in this section, was again compared with that of hybrid II. Although the actual number of univalents accumulated at the plate at each stage was very similar for both hybrids, the proportions of these in terms of the average of the total number of univalents were lower in hybrid II than in hybrid I (Table VI). The univalents of the less irregular cells also tended to accumulate a little faster than those of the more irregular cells. When the accumulation of univalents for the cell categories was plotted in the same way as in Fig. 11 for data of hybrid I, similar concave curves were obtained. In general the behavior of the univalents in hybrid II corresponded closely to that in hybrid I.

3. Behavior of the Univalents at Anaphase I and Telophase I

In the preceding sections the theory was advanced that the least irregular metaphase I cells tend to enter anaphase I sooner than the more irregular

cells. If this hypothesis is correct, then anaphase I and telophase I cells observed in L anthers should, as a consequence, show fewer lagging univalents then the anaphase I and telophase I cells in the VL anthers (see Fig. 3). One may even expect a similar difference in lagging between anaphase I and telophase I cells in L and in VL anthers. Telophase I cells in these anthers should then have a somewhat lower average of univalents lagging at the equator than anaphase I cells.

To test this hypothesis 334 anaphase I and telophase I cells of hybrid I were grouped separately according to their stages L and VL, as shown in Fig. 12 and Table VIII. As anticipated, more lagging chromosomes were found in cells at VL than in those at L. Consequently, the whole frequency curve of the VL stage has shifted toward the side with higher numbers of laggards (Fig. 12).

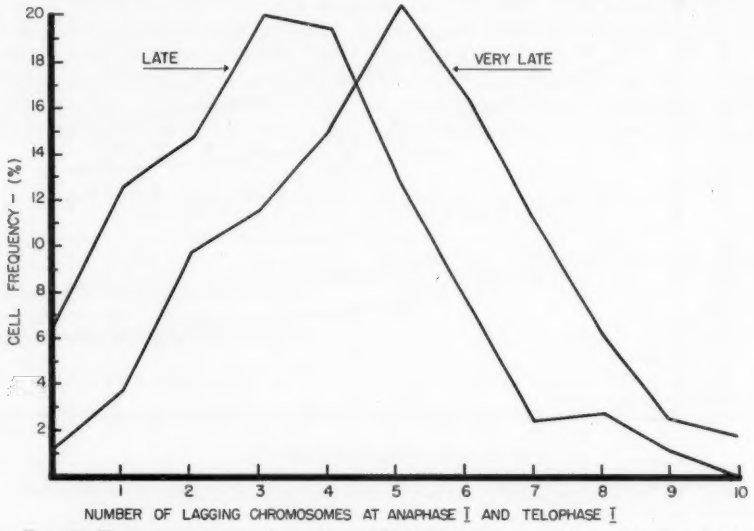


FIG. 12. Frequency curves of numbers of lagging chromosomes in anaphase and telophase cells in late and very late anthers of hybrid I.

TABLE VIII

Averages of numbers of laggards observed in anaphase I and telophase I cells in L and VL stages in hybrid I and hybrid II

		Late			Very late		
	Hybrid	AI	TI	Total	AI	TI	Total
No. cells analyzed	I	81	89	170	84	80	164
Average no. of laggards	I	3.60	3.22	3.40	4.85	4.90	4.87
No. cells analyzed	II	45	51	96	29	29	58
Average no. of laggards	II	5.71	3.96	4.78	7.04	5.90	6.46

Table VIII shows that the anaphase and telophase cells at VL of hybrid I had on the average 1.47 more laggards than those at L, while hybrid II had 1.68 more laggards at VL than at L.

A similar situation can be seen when comparing the averages of anaphase cells with those of telophase cells in the L stage of both hybrids and in the VL stage of hybrid II. The averages were generally lower in the telophase cells than in the anaphase cells. These findings are thus consistent with the hypothesis that the least irregular cells pass earlier into anaphase I than the more irregular cells.

The next question is, can the amount of lagging observed in these anaphase I and telophase I cells be measured? It was found that during metaphase I of hybrid I up to 67% of the univalents accumulated at the equatorial plate. At the onset of anaphase I these univalents, due to their positions, orient and become the laggards in anaphase and telophase cells (Figs. 6, 7, 8, and 9; refs. 10, 13, 15, 16). Yet, the univalents at the equator of the observed metaphase I cells of the M stage (the representative stage) cannot be compared with the laggards in the telophase and anaphase cells, for the latter cells have been derived from only a part of the metaphase cells in M, in fact, from the most regular part of the metaphase cell population (Fig. 13). Since this portion of

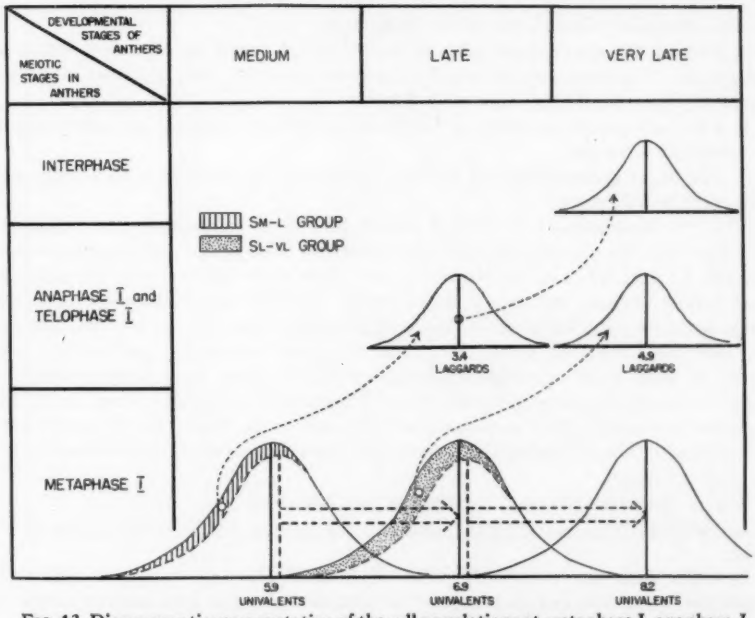


FIG. 13. Diagrammatic representation of the cell populations at metaphase I, anaphase I, and telophase I, and interphase, observed in anthers of *T. timopheevi* × *T. durum* hybrids in the three developmental stages medium, late, and very late. Arrows show the approximate development of the populations from medium to very late. Solid lines enclose observed populations, while shaded area hypothetically shows the S groups. Population sizes are taken arbitrarily and do not represent the actual proportions.

cells is unknown and since the number of univalents at the plate in the M stage was only a fraction of the maximum accumulated during metaphase I, a direct relation between univalents at the plate of the observed metaphase I cells and the laggards at anaphase I and telophase I does not exist. However, there exists a direct relationship between those metaphase cells of the M stage that proceeded, as the earliest group, into anaphase I, and the anaphase and telophase cells observed at the L stage (Fig. 13). The latter cells are, in fact, the same cells as the former. It is impossible, however, to derive from anaphase I and telophase I cells the chromosome associations they had at metaphase I, although an estimate of this group of metaphase cells would be very useful. It would not only provide a means to measure with reasonable accuracy the degree of lagging of the univalents, but also to give a picture of the behavior pattern of the cells in transition from metaphase I to anaphase I in these *T. timopheevi* × *T. durum* hybrids. However, a method was found for making this estimate on the basis of the data available from the metaphase I cells. The method and calculation of the degree of lagging at anaphase I and telophase I will be discussed in the following sections.

Analysis

1. The Statistical Calculation of the Subgroups

A part of the metaphase cells of each hybrid could be classified into two subgroups (S groups), according to the time at which they proceed into anaphase I (Fig. 13). These were as follows:

1. The $S_{M\text{-}L}$ group occurred at the M stage and, on passing into anaphase I, induced the L stage.

2. The $S_{L\text{-}VL}$ group occurred at the L stage and, on passing into anaphase I, induced the VL stage.

The cell categories of the two S groups and their frequencies are unknown, but they can be calculated from the observed metaphase cell populations of the M, L, and VL stages. However, for these calculations only populations that follow normal frequency distributions can be used. Therefore, the raw data, as shown in Table I, for the cell populations of M, L, and VL are not suitable. As shown in Table III, each observed metaphase population consisted of four smaller subpopulations, of which three were large enough to show a normal frequency distribution. These subpopulations were, therefore, regarded as independent populations from which the respective S groups were calculated. In the following the S groups of these subpopulations will be referred to as S′ groups.

The subpopulations used for the calculations were:

1. Cells without a trivalent, that is, cells having bivalents and univalents only.

2. Cells with one and with three trivalents. The two types of cells were combined into one group because the number of cells with three trivalents was very small.

3. Cells with two trivalents.

There were thus nine subpopulations, three for each of the developmental stages M, L, and VL. After the appropriate calculations the six S′ groups,

derived from the nine subpopulations, were recombined to provide the two S groups $S_{M\text{-}L}$ and $S_{L\text{-}VL}$ that were referred to earlier.

The procedure for calculating the S groups was as follows. Firstly for each of the nine subpopulations the theoretical normal frequency curve was calculated. This was done by calculating the c-values[3] of each of the subpopulations, after which the theoretical frequencies could be determined. Then the S′ group of each subpopulation was determined. Assume that in Fig. 14 curve M represents the frequency curve of one of the subpopulation at M, and L that of the same subpopulation at L. The S′ group that caused the differences between these two populations can be estimated by superimposing a point P, located on the M curve, on point P', which is located on the L curve. P and P' should be as far to the right as possible on the frequency curves. The factor with which the value of P was multiplied to give the value of P' can now be used as a constant multiplier for each class of subdivision M to give curve M′. The differences between the classes of the M′ and L subpopulations (shaded area in Fig. 14), when expressed in percentages, is the required estimate of the $S'_{M\text{-}L}$ group of the subpopulation concerned.

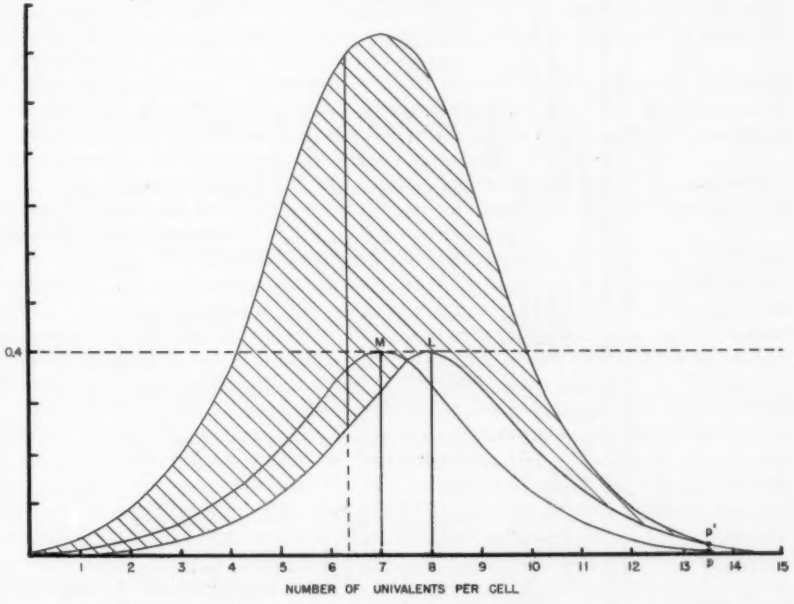


FIG. 14. Frequency curves of metaphase cell populations in the medium and late stage. Shaded area, when converted to percentages, represents the $S_{M\text{-}L}$ group.

[3]The formula $c=(X-\bar{x})/s$ was used, in which X is the class expressed in the number of univalents, $\bar{x}$ the estimate of the population average, s the standard deviation, and c the normal variate (a standard measure). The ordinates of the normal curve are taken from Goulden, Table A-2, p. 442 (6).

TABLE IX

Calculated normal populations of the three subdivisions of both hybrids shown for each of three developmental stages, M, L, and VL and their respective S groups. Cell categories are expressed in univalents per cell and their frequencies in percentages

Total no. univalents per cell category	Subpopulation No. 1: no trivalents					Total no. univalents per cell category	Subpopulation No. 2: 1 and 3 trivalents per cell					Total no. univalents per cell category	Subpopulation No 3: 2 trivalents per cell				
	M	S'_{M-L}	L	S'_{L-VL}	VL		M	S'_{M-L}	L	S'_{L-VL}	VL		M	S'_{M-L}	L	S'_{L-VL}	VL
							A. Hybrid I										
0	1.0	0.9	1.2	1.5	0.2	1	5.8	12.0	2.1	2.6	0.3	0	2.3	2.8	1.2	2.3	0.5
2	4.9	5.0	4.7	5.6	1.0	3	16.8	28.7	9.6	11.6	3.0	2	12.7	14.7	8.0	12.6	4.9
4	14.9	15.2	12.1	14.2	4.5	5	*28.3*	*35.0*	24.1	27.4	12.9	4	30.9	*33.4*	24.7	30.3	20.9
6	26.4	26.9	21.3	23.6	12.3	7	27.4	20.1	*31.8*	*32.8*	28.5	6	*33.7*	33.1	*35.1*	*32.2*	*37.1*
8	*27.7*	*28.0*	*25.1*	*25.9*	22.2	9	15.6	4.2	22.4	19.6	*31.6*	8	16.4	13.7	23.0	16.7	27.2
10	17.2	17.0	20.0	18.4	*26.1*	11	5.1	—	8.3	5.5	17.8	10	3.6	2.2	7.0	6.0	8.3
12	6.3	5.9	10.7	8.3	19.9	13	1.0	—	0.6	5.0	5.0	12	0.4	0.1	1.0	0.8	1.0
14	1.4	1.1	3.9	2.3	9.9	15	0.1	—	0.2	—	0.7	14	—	—	0.1	—	0.1
16	0.2	0.1	0.9	0.3	3.2	17	—	—	—	—	0.1	16	—	—	—	—	—
18	—	—	0.2	—	0.7	19	—	—	—	—	—	18	—	—	—	—	—
20	—	—	—	—	0.1	21	—	—	—	—	—	20	—	—	—	—	—
							B. Hybrid II										
0	0.3	0.4	0.2	0.2	—	1	1.8	3.4	0.2	0.2	0.4	0	0.1	0.1	0.1	0.1	—
2	1.5	1.8	0.8	0.8	0.2	3	6.8	12.7	0.6	0.4	2.0	2	1.8	2.0	1.2	1.3	0.2
4	5.0	5.7	3.0	3.0	1.3	5	16.3	25.0	7.1	7.2	6.5	4	13.2	14.4	9.1	9.3	4.3
6	11.8	13.2	8.0	8.0	5.7	7	*25.2*	*30.8*	19.2	19.7	14.9	6	*35.1*	*37.1*	28.5	29.0	16.1
8	19.8	21.3	15.6	15.6	15.3	9	25.0	20.8	*29.5*	*30.2*	22.9	8	35.0	34.4	*36.8*	*37.1*	*30.8*
10	*23.5*	*24.2*	21.5	21.5	25.5	11	16.0	6.7	25.9	26.1	*24.1*	10	12.9	10.9	19.6	19.2	29.8
12	19.7	18.8	*22.1*	*22.1*	*26.3*	13	6.6	0.6	13.2	12.6	17.3	12	1.8	1.0	4.3	3.9	14.7
14	11.7	10.1	16.1	16.1	16.9	15	1.8	—	3.8	3.3	8.4	14	0.1	—	0.4	0.3	3.7
16	4.9	3.6	8.5	8.5	6.8	17	0.3	—	0.6	0.4	2.8	16	—	—	—	—	0.5
18	1.4	0.8	3.2	3.2	1.7	19	0.1	—	0.2	—	0.6	18	—	—	—	—	—
20	0.3	0.1	0.9	0.9	0.3	21	—	—	—	—	0.1	20	—	—	—	—	—
22	0.1	—	0.2	0.2	—	23	—	—	—	—	—	22	—	—	—	—	—

The calculated normal populations of the subpopulations together with their S′ groups, shown for the M, L, and VL stages of both hybrids, are presented in Table IX. This table shows that the values of the classes of the S′ groups of subpopulations 1 and 3 are only slightly, but consistently, different from those of their respective subpopulations. The number of univalents in these S′ groups are only slightly less compared to those of their subpopulations. An exception to this behavior is the $S_{L\text{-}VL}$ group of subpopulations No. 1 in hybrid II. Since the estimates of the averages of this subpopulation at L and VL are almost the same, the $S_{L\text{-}VL}$ group is kept identical with the subpopulation at L, despite some differences in the standard deviations. The greatest differences between the S′ groups and their respective subpopulations are found in subpopulation 2, which contains mostly cells having one trivalent. Here the $S'_{M\text{-}L}$ group is much less irregular than the subpopulation at M. In hybrid I the cell categories with 11, 13, and 15 univalents per cell are not represented in this S′ group, while in hybrid II the cell categories 15, 17, and 19 were lacking. These differences become smaller when one compares the $S'_{L\text{-}VL}$ group with the subpopulation at L. The S′ groups, as shown in Table IX, thus confirm the expectation, as mentioned before, that the least irregular metaphase I cells have, at first, the greatest chance to proceed into anaphase I, leaving, in the metaphase I cell populations, larger proportions of more irregular cells.

The strongly pronounced trend in the S′ groups of subpopulation 2 in both hybrids may have been due to a different expression of the developmental rates of the various cell categories in this subpopulation compared to the others. As a consequence two things may have happened:

1. The onset of anaphase I was slightly later for the whole subpopulation, in which each cell had one trivalent, than for the cell categories belonging to other subpopulations.

2. Although the least irregular cells of this subpopulation proceeded into anaphase I at approximately the same time as those of the other subpopulations, the more irregular the cells, the more progressively prolonged the duration of metaphase I became. For these cells the onset of anaphase I thus started later than for comparable cell categories of the other subpopulations. Of the two situations mentioned point 1 is arbitrarily included in Fig. 3.

To synthesize the three entire S groups of both hybrids so that they include all cell categories, the three S′ groups of each developmental stage were combined (Table IX). For this each S′ group was assigned a frequency that closely approached the actual value. To obtain the theoretical frequencies of cell categories of the main populations at M, L, and VL of both hybrids the subpopulations (Table IX) were similarly combined according to their true proportions (see Table III). These metaphase populations and their S groups thus calculated from the data of both hybrids are shown in Table X.

It is emphasized that all frequencies of the cell categories of the S groups are expressed in percentages. No estimates of the actual population sizes can be made. With these calculated S groups, in which the metaphase cells are classified according to the number of univalents per cell, the way is open to measure the total amount of lagging as observed at anaphase I and telophase I.

TABLE X

Theoretical frequencies of metaphase I cell categories, expressed in percentages, and their respective S groups in the three developmental stages M, L, and VL

Total no. univalents per cell category	Hybrid I					Hybrid II				
	Medium	S_{M-L}	Late	S_{L-VL}	Very late	Medium	S_{M-L}	Late	S_{L-VL}	Very late
0	0.5	0.6	0.4	0.5	0.1	0.1	0.1	—	—	—
1	4.2	8.7	1.5	1.9	0.2	1.2	2.3	0.1	0.1	0.3
2	2.8	3.0	1.9	2.7	1.1	0.5	0.6	0.3	0.3	—
3	12.1	20.9	6.8	8.5	2.0	4.6	8.6	0.4	0.3	1.5
4	7.1	7.2	5.5	6.6	4.5	2.6	2.9	1.6	1.7	0.6
5	*20.3*	*25.6*	17.0	20.0	8.7	11.0	17.0	4.7	4.9	5.0
6	8.8	8.3	8.4	7.8	8.6	6.6	7.1	4.8	5.1	2.3
7	19.7	14.7	*22.5*	*23.9*	19.3	*16.9*	*20.9*	12.6	13.4	11.4
8	5.8	5.1	7.0	5.4	8.1	8.3	8.4	7.5	7.6	5.1
9	11.2	3.1	15.9	14.3	*21.4*	16.8	14.2	*19.4*	*20.6*	17.7
10	2.4	2.1	3.8	2.7	5.1	6.5	6.2	7.2	6.6	6.3
11	3.6	—	5.9	4.0	12.1	10.7	4.6	17.0	17.8	*18.6*
12	0.7	0.6	1.6	1.0	2.9	4.4	3.9	5.8	4.9	4.9
13	0.7	—	1.1	0.4	3.4	4.5	0.4	8.6	8.6	13.3
14	0.1	0.1	0.6	0.2	1.4	2.5	2.0	3.9	3.3	2.6
15	0.1	—	0.1	—	0.5	1.2	—	2.5	2.2	6.5
16	—	—	0.1	—	0.4	1.0	0.7	2.1	1.7	0.9
17	—	—	—	—	0.1	0.2	—	0.4	0.3	2.1
18	—	—	—	—	0.1	0.3	0.2	0.8	0.6	0.2
19	—	—	—	—	—	—	—	—	—	0.5
20	—	—	—	—	—	—	—	0.2	0.2	—
21	—	—	—	—	—	—	—	—	—	0.1

2. The Amount of Lagging at Anaphase I and Telophase I

Before describing the measurement of the degree of lagging of the univalents at anaphase I and telophase I, it should be pointed out that for the most reliable calculations only the S_{M-L} group and the anaphase and telophase cells of the L anthers could be used because the latter cells originated directly from the S_{M-L} group (Fig. 13). The anaphase I and telophase I cells of the VL anthers can be compared with the S_{L-VL} group, although the error in calculating from this group the degree of lagging in VL anthers may be somewhat greater than that in the calculations involving the S_{M-L} group. The estimate of the amount of lagging in the VL anthers may, however, be of importance in substantiating the amount of lagging calculated for the L anthers.

The lagging of univalents can be measured by the statistical treatment of the number of univalents of the cell categories of the S_{M-L} group. If one assumes a 50% chance of lagging for all univalents, the ratio of lagging at anaphase I, expressed in percentages, can be obtained by expansion of the binomial $f.\ (1/2 + 1/2)^n$, in which n is the number of univalents and f the frequency of the cell categories of the S group. The same procedure can be followed for calculating the ratios of lagging for an assumed chance of lagging of the univalents of 66.7% (expansion of $f.(1/3 + 2/3)^n$) and 75% (expansion of $f.\ (1/4 + 3/4)^n$). Using only the S_{M-L} group of hybrid I three ratios of lagging for anaphase I were obtained (Table XI). By means of the chi-square method these ratios were tested for goodness-of-fit with the lagging observed in anaphase and telophase cells of the L anthers (Table XII).

The results show that the percentage of lagging lay between 66.7 and 75% and was probably very near 70%. This figure can be substantiated by another, simple calculation. The average number of univalents per cell of all categories in the S_{M-L} group of hybrid I was 4.86, whereas in the anaphase and telophase

TABLE XI

Calculation of lagging ratios expected in anaphase I and telophase I based on metaphase cells of the $S_{w.t.}$ group of hybrid I for lagging chances per univalent of 50%, 66.7%, and 75%

Total no. univalents per cell category	Cell frequency of $S_{w.t.}$ group (%)	Expected number of lagging univalents at anaphase I and telophase I											Total %
		0	1	2	3	4	5	6	7	8	9	10		1	
A. Assumed lagging 50%*															
0	0.57	0.57													
1	8.74	4.37	4.37												
2	3.00	0.75	1.50	0.75											
3	20.94	2.62	7.85	7.85	2.62										
4	7.20	0.45	1.80	2.70	1.80	0.45									
5	25.55	0.80	3.99	7.98	7.98	3.99	0.80								
6	8.32	0.13	0.78	1.95	2.60	1.95	0.78	0.13							
7	14.69	0.12	0.59	2.47	4.12	4.12	2.47	0.59	0.12						
8	5.13	0.02	0.16	0.56	1.12	1.40	1.12	0.56	0.16	0.02					
9	3.07	0.01	0.05	0.22	0.50	0.76	0.76	0.50	0.22	0.05	0.01				
10	2.07	—	0.02	0.99	0.24	0.42	0.51	0.42	0.24	0.09	0.02	—			
11	—	—	—	—	—	—	—	—	—	—	—	—		—	
12	0.61	—	—	0.01	0.03	0.07	0.12	0.14	0.12	0.07	0.03	0.01		—	
13	—	—	—	—	—	—	—	—	—	—	—	—		—	
14	0.11	—	—	—	—	0.01	0.01	0.02	0.03	0.02	0.01	0.01		—	
	Total	9.84	21.11	24.58	21.01	13.17	6.57	2.36	0.89	0.25	0.07	0.02		—	99.87
Expected lagging ratio		9.8	21.1	24.6	21.0	13.2	6.6	2.4	0.9	0.3	0.1	—		—	100.0
B. Assumed lagging 66.7%†															
Expected lagging ratio		4.8	13.8	18.3	21.7	17.7	12.4	6.5	3.1	1.1	0.4	0.1		—	99.9
C. Assumed lagging 75%‡															
Expected lagging ratio		3.3	11.4	14.8	20.7	18.0	15.2	8.7	4.8	1.9	0.8	0.3		0.1	100.0

*Expansion of formula $f_n\ (1/2 + 1/2)^n$, in which f is the cell frequency and n the number of univalents of the cell categories.
†Expansion of formula $f_n\ (1/3 + 2/3)^n$.
‡Expansion of formula $f_n\ (1/4 + 3/4)^n$.

TABLE XII

Chi-square test of lagging obsreved at anaphase I and telophase I of L stage of hybrid I and lagging calculated from $S_{M\text{-}L}$ group at metaphase I, assuming lagging probabilities of 50%, 66.7%, and 75% per univalent

	No. cells	Number of laggards per cell											
		0	1	2	3	4	5	6	7	8	9	10	11
A. Lagging probability of 50%													
Observed lagging	170	11	21	24	35	33	22	13	4	5	2	—	—
Expected lagging	170	16.7	35.9	41.8	35.7	22.4	11.2	4.1	1.5	0.5	0.2	—	—
		For classes 0–5* : $\chi^2 = 30.39$; d.f. = 5; $P < 0.01$											
		For classes 0–9 : $\chi^2 = 110.58$; d.f. = 9; $P < 0.01$											
B. Lagging probability of 66.7%													
Observed lagging	170	11	21	25	34	33	22	13	4	5	2	—	—
Expected lagging	170	8.2	23.5	31.1	36.9	30.1	21.1	11.1	5.3	1.9	0.7	0.2	—
		For classes 0–7* : $\chi^2 = 3.94$; d.f. = 7; $P \sim 0.75$											
		For classes 0–10 : $\chi^2 = 9.33$; d.f. = 10; $P \sim 0.50$											
C. Lagging probability of 75%													
Observed lagging	170	11	21	25	34	33	22	13	4	5	2	—	—
Expected lagging	170	5.6	19.4	25.2	35.2	30.6	25.8	14.8	8.2	3.2	1.4	0.5	0.2
		For classes 0–7* : $\chi^2 = 8.50$; d.f. = 7; $P \sim 0.30$											
		For classes 0–10 : $\chi^2 = 10.47$; d.f. = 11; $P \sim 0.50$											

*Classes which have an expected frequency of 5 or more.

cells at L there were an average of 3.40 lagging univalents per cell, which is 70.1% of all univalents present. It can be seen from Table XII that for the 170 cells the expected lagging ratio of 70% must be very similar to the observed one.

The average amount of lagging at VL, calculated from all cell categories of the S_{L-VL} group, was 76.4%. This value is close to the 70% calculated for the anthers at the L stage. The degree of lagging was apparently very similar for the slightly irregular, as well as for the very irregular, cell categories during all stages of development in the metaphase I – anaphase I transition of hybrid I. Such a conclusion would be of great importance and the calculations were, therefore, also made with the data of hybrid II. These calculations showed that lagging in the anaphase and telophase cells in the L stage of hybrid II amounted to 66.8%, whereas in the VL stage these cells had a lagging of 67.3%. These two values are very close indeed, although different groups of cells were involved in the calculations. They thus substantiate the finding in hybrid I, and the conclusion may be drawn that in both hybrids the degree of lagging is approximately the same for all metaphase cell categories as they pass into anaphase I.

It is well known that univalents which lie in the equatorial plate at the end of the metaphase I will become the laggards at anaphase I (13, 15, 16). The number of laggards at anaphase I should thus be very similar to the number of univalents accumulated at the equatorial plate during metaphase I. It was found that approximately 70% of the total number of univalents lagged at anaphase I and telophase I of hybrid I at L. These anaphase and telophase cells, however, originated directly from S groups, which were portions of the observed metaphase cell population at M and L, each of which had an average of 23.7% and 46.4%, respectively, of univalents accumulated at the equator (Table VI). There is thus a considerable discrepancy between these latter values and the amount of lagging at anaphase I and telophase I. When considering the data of hybrid II, similar discrepancies are evident. There the degree of lagging in anaphase and telophase cells at L and VL was approximately 67% but the metaphase cells at M and L, from which they partly originated, had on the average only 13.3% and 37.4%, respectively, of the univalents on the plates (Table VI). The question of how to account for these discrepancies can be raised.

It was suggested above that the degree of lagging was the same (approximately 70%) for all cell categories at metaphase I for both hybrids, when they proceeded into anaphase I and telophase I. This important feature could be deduced from the findings that the degree of lagging was very similar at L and VL, and that the expected lagging ratios, calculated from the S groups for a probability of univalent lagging of 66.7 and 75%, were very close in all classes to the observed lagging at anaphase I and telophase I (Table XII). This suggests that there was a tendency of the metaphase cells to proceed into anaphase I when approximately 70% of the univalents were on the plate. It seems likely that before passing into anaphase I, metaphase cells attain a state of equilibrium. The time required to reach this equilibrium is probably closely connected with the total number and behavior of the univalents, the number of univalents at the plate and the presence of trivalents. It may be possible that in most metaphase cells of these two hybrids the specific equilibrium

necessary for further development will usually be reached when approximately 70% of the univalents have accumulated at the plate. It can then be assumed that the anaphase and telophase cells of the L and VL stages were derived from metaphase cells in the M and L stages, respectively, which had already accumulated approximately 70% of their univalents in the equatorial region. Since the average accumulation of univalents at the plate for the total metaphase population at M and L was much lower, it can be concluded that at first only a very few cells with the highest numbers of univalents at the plate (approximately 70%) passed into anaphase I and that the transition rate was slow. This transition rate would speed up gradually as more univalents accumulated at the plates and more and more cells would fulfill the conditions required for equilibrium.

In the above it is suggested that all cell categories at metaphase I will eventually pass into anaphase I when approximately 70% of the univalents have accumulated at the plates, including those cells still at metaphase I at VL. If this is so, then there is a small discrepancy between the amount of lagging at anaphase I and telophase I (70%) and the highest averages (55 to 65%) of the univalent accumulation at the plates found in some cell categories at VL. The following three causes may have induced these apparent discrepancies:

1. The percentages of accumulation of the univalents were calculated as *averages* for the cell categories, which contained metaphase cells that were just about to proceed into anaphase I (maximum number of univalents at the equatorial plate) as well as cells not yet so far advanced (lower number of univalents at equator). As pointed out above, the cells with most univalents on the plates proceeded first into anaphase I. It is thus reasonable to assume that the average number of univalents at the plate at the critical period for the metaphase cells to pass into anaphase I must be higher than the highest *average* of univalent accumulation found in the VL anthers.

2. At the time of the onset of anaphase I other univalents located close to the equatorial region may also have lagged, while the bivalent partners separated and moved towards the poles. These laggards would not necessarily orient and split. Such unsplit univalents were sometimes noticed between the plate and one of the poles. Although a few were recognized, others may have been recorded as oriented laggards.

3. It has been reported that multivalents may be a source of additional lagging chromosomes (3, 16, 17). Since the *T. timopheevi* hybrids regularly formed trivalents, the possibility that these trivalents were responsible for some extra laggards cannot be dismissed.

It is very probable that points 2 and 3 have not added significantly to lagging and that their effect was, therefore, small.

Discussion

The cytological data that were presented in the preceding sections showed that a specific pattern of behavior of the pollen mother cells occurred at the time of the transition from metaphase I to anaphase I in the developing anther. In general, the least irregular metaphase I cells developed more quickly through metaphase I than the more irregular cells. This behavior is plausible since the

least irregular cells more closely approached the stable and most efficient condition of cells, namely, having bivalents only. The greater the meiotic irregularities, the longer it takes the cell to stabilize and to proceed into anaphase I. It was suggested, therefore, that this specific behavior of the metaphase I cells is chiefly the result of differences in the rate at which the cells reach a certain state of equilibrium among certain cellular forces present which may trigger the start of anaphase I. The degree of efficiency of the pollen mother cells in attaining this equilibrium is apparently dependent to a great extent on the number of univalents and the presence of trivalents. However, it seems to be independent of the degree of terminalization of the chiasmata, since the terminalization coefficients in M and L stages were equal. Based on the results of the present studies it is difficult to suggest what conditions lead to such an equilibrium in irregular cells. It is still possible, however, to make some speculations when considering some of the observations that have been made by others in animal cells.

Specially treated neuroblasts of grasshoppers often contained a chromosome which was attached to the spindle near one of the poles (2, 5). It was observed that anaphase did not begin until this chromosome had reached the metaphase plate. Carlson (2) concluded that the spindle force is a single unit separating simultaneously all sister chromatids and that its action depends on a certain "state of order" to initiate anaphase. This "state of order" is probably an equilibrium of cellular forces which must be attained before chromosome division can occur. It is conceivable that certain forces which control the mitotic behavior of the chromosomes also play an important role in controlling meiotic cell division. If this is so the observations of the above-mentioned workers may fit into the pattern found in the first meiotic division of the *T. timopheevi* hybrids. In normal tetraploid *T. timopheevi* or *T. durum* certain cellular forces are satisfied when 14 bodies, in the form of 14 bivalents, are coöriented at the equatorial plate. Because the state of equilibrium is easily attained in these cells, metaphase I is of short duration. In the *T. timopheevi* × *T. durum* hybrids, in which the meioses are irregular and which usually contain five to nine univalents per cell, the same cellular forces also control cell division. However, these cells rarely form 14 bivalents and they require, therefore, a longer time to reach a state of equilibrium. To attain this equilibrium, the deficit of bivalents may be eliminated by the cells through "filling up" the equatorial plate with univalents. By doing so the requirement for cell division of 14 bodies at the plate may thus be more or less fulfilled. Hereafter, when all cellular forces reach a certain equilibrium, anaphase I can set in. In the *T. timopheevi* hybrids it was found that anaphase I was not initiated unless approximately 70% of the univalents reached the equatorial plate, so that a total of slightly more than 14 chromosomal bodies, the number apparently required by the cells, were present at the equator. A univalent can, therefore, apparently substitute partly for a bivalent so that an approximate fulfillment is achieved of the conditions that trigger the onset of anaphase I. Such a mechanism would explain why terminalization of chiasmata does not alone play a role in these hybrids; the equilibrium of cellular forces that must be reached first apparently depends greatly on the number and the kind of chromosomal bodies (univalents, bivalents, trivalents, etc.) at the equatorial plate.

With the above hypothesis we can now explain the observed pattern of development during the metaphase I – anaphase I transition. The average percentages of univalents accumulated at the equatorial plate in the cell categories of the three developmental stages M, L, and VL are shown in Fig. 15, which is an adaptation of Fig. 10. In Fig. 15 the population curves for the three stages are concave, which indicates that the univalents of the least irregular cells reach the equator slightly earlier than those of the more irregular cells. These first cells will, therefore, approach the critical univalent accumulation of 70% earlier than the more irregular cells, giving them an advantage for proceeding into anaphase I. Eventually, the most regular cell categories would have moved out of the population leaving the more irregular cells at metaphase I, as indicated by curves 3 and 4. Curve 4 hypothetically represents the probable metaphase cell population in the very latest developmental stage just before proceeding into anaphase I.

If the above hypothesis is basically true then one should not regard the critical univalent accumulation of 70% as the moment at which *every* metaphase cell will proceed into anaphase I. There are probably several factors in a cell controlling the onset of anaphase I; the requirement of a certain number of chromosomal bodies in the plate may be only one of them. When these

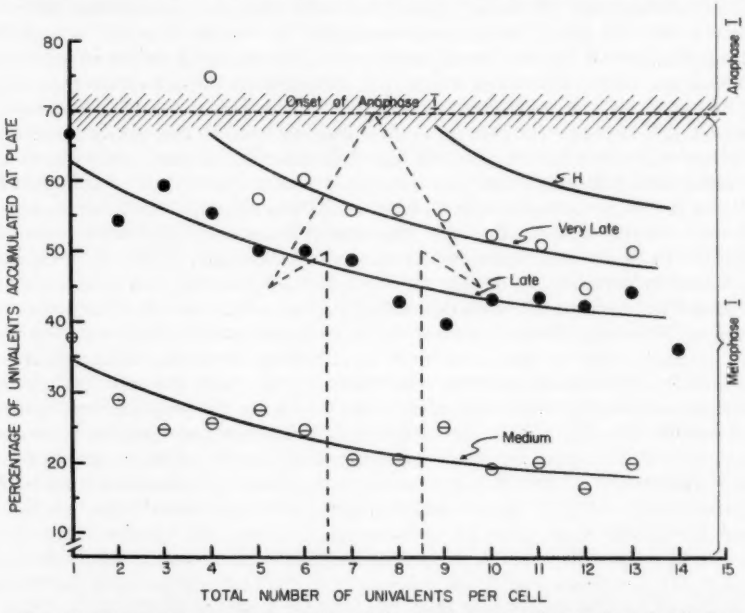


FIG. 15. Diagrammatic representation of the development of metaphase I cells based on the accumulation of univalents at the equatorial plate for the three stages medium, late, and very late (curves 1, 2, and 3, respectively). Curve 4 represents a hypothetical, still later stage. The arrow indicates the direction of the development, while the broken line at 70% univalent accumulation indicates the approximate moment when the metaphase I cells proceed into anaphase I.

cellular factors attain a harmonious state anaphase I is initiated just before or some time after the moment that a cell has 70% of its univalents on the equator. Furthermore, most univalents of a cell move towards the equator, and several of them may reach it simultaneously. This, together with the fact that each univalent of the cells with the least total number of univalents represents a significant proportion of the univalents, may result in the accumulation of more than 70% of the univalents at the plate before cell division actually takes place.

The trend towards greater irregularity in metaphase populations during the progression of the transition from metaphase I to anaphase I can thus be satisfactorily explained by (1) the tendency of the univalents of the least irregular cells to accumulate a little faster at the plates than the univalents of the more irregular cells, when considered in relation to (2) the hypothesis that cells need a specific number of chromosomal bodies on the plate for an efficient development of cell division. Point 1 is an observed fact, although it is difficult to explain. This problem is presently under investigation. Point 2 is a speculative one, although findings reported here and elsewhere (2, 5) indicate it as one of the determining factors in cell division. Without taking this point into consideration, the observed trends in the metaphase cell population of developing anthers would be very difficult to explain. This hypothesis should, however, be regarded as tentative until comparable data of other hybrids are available. Closely connected with this problem is the question of how common is the phenomenon of the shift to greater irregularity during development of metaphase I. The writer has obtained evidence (unpublished) that these trends are limited to certain plants or hybrids. Other hybrids do not show this phenomenon at all, and the metaphase I populations during their transition into anaphase I remain constant in these hybrids, indicating that all cell categories simultaneously produce anaphase I cells at a similar rate. The factors governing these different kinds of developmental behavior of metaphase cells are presently under investigation and will be discussed in a future publication.

This study clearly demonstrated that the trend towards greater irregularity in the later developmental stages was not due to environmental differences to which the anthers may have been exposed. If the environment had induced the differences that were found, the strikingly *regular* trends shown in Fig. 2 and Table II for the metaphase cells and in Fig. 11 and Table IX for the anaphase and telophase cells would not have taken place. In the past much research devoted to studies on the effects of environmental conditions on the meiosis have been published. Striking results were reported, particularly in those cases where plants were exposed to extreme conditions of temperature and drought (1, 4, 11, 18, 19, 21). The reports on the effects of average field conditions on meiosis are much less convincing; in fact, Mather (14) and Oehlkers (18) found no environmental effects on plants grown under such circumstances. However, cytological differences among fixing dates were reported by many workers, as, for example, Kihara (12), Katayama (11), Hosono (9), Sears (20), and Grun (7). Although these differences may have indeed been due to differences in environmental conditions at the moment of fixation of the pollen mother cells, they may have equally been due to a developmental effect of the type described in the present paper.

It is thus clear that in cytological studies on chromosome associations the stage of metaphase I must be taken which is representative for the plant involved, particularly if a behavior pattern similar to that described in this paper is found. In such cases anthers containing only metaphase cells (medium stage) should be analyzed.

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INFECTION OF SCOTS PINE ROOTS BY FOMES ANNOSUS[1]

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Abstract

A method for *in situ* inoculation of Scots pine roots with *Fomes annosus* is described. Replacement of the fungus within the inoculum and rate of movement of the fungus on to the root surface were two important considerations in the development of a successful inoculation technique. Preliminary observations on host vigor, food-base size, and resin production as factors influencing growth of *F. annosus* are noted.

In laboratory and field trials, spores of *Fomes annosus* caused infection of Scots pine roots only when sown on woody tissues exposed by cutting. A method is described whereby it was possible to observe development of spores on the surface of pine roots. The influence of microbiological and nutritional factors on infection of roots by *F. annosus* spores is discussed.

Artificial Inoculations

Fomes annosus (Fr.) Cke., a common root-rotting fungus, reveals its presence in a tree by aboveground symptoms only after the disease is in an advanced stage. Use of natural infection in studying the biology of the fungus is time-consuming and has only limited application. Artificial inoculations must be used where it is desired to control the time and point of initial infection and where existing environmental conditions require modification.

In the past, attempts to inoculate coniferous hosts with *F. annosus* have met with limited success. Agar, seed, and sawdust cultures when in contact with soil are extremely susceptible to colonization by other microorganisms. Pine roots, closely approximating to the natural habitat, are a suitable substrate for growing *F. annosus*. However, Rishbeth (unpublished) found that colonization of naturally infected root lengths by secondary fungi reduced the number of successful infections below a practical level. Branches of beech (*Fagus sylvatica* Linn.) proved to be a satisfactory substrate for maintaining the fungus in a viable state during trials lasting 18 months.

Beech branches infected with *F. annosus* were used as inoculum in the current investigation to furnish information about fungal development under various conditions. The final technique adopted and results of preliminary investigations into host vigor and food-base requirements are reported here.

Initially the following technique was employed: beech branches 3–4 cm in diameter and 4–6 cm long were sterilized in an autoclave at 20 lb pressure for two periods each of 60 minutes, 24 hours elapsing between each period. The blocks were then inoculated with a sawdust culture of *F. annosus*, and incubated for 4 months at 22° C. At the end of this time the fungus had

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penetrated the wood but no advanced decay was evident. Bark was retained on the blocks since there was some evidence that it retarded rapid replacement of *F. annosus* by other organisms. Blocks were wired in position with the bark contacting the pine roots. One hundred and twelve inoculations were undertaken to test the influence of host vigor, site, and size of food base on the rate of *F. annosus* infection after 5–12 months. The majority of these inoculations failed, making it necessary to devise other inoculation techniques before undertaking further experiments.

The following points were evident from the preliminary trials: (1) beech branches, when prepared as described, served as a suitable substrate for keeping *F. annosus* in a viable state for at least 12 months; (2) in a few instances where the site of inoculation had been covered with polythene sheeting the fungus produced a heavy mat of mycelium on the exposed wood surfaces, but this did not occur when soil was placed in direct contact with the inoculum; (3) wiring inoculum blocks in position maintained a close contact between the fungus and root but tended to cause some injury when left for long periods on fast-growing roots; (4) mycelium was apparently able to emerge from the periphery of the beech blocks only where the bark was cracked or otherwise disrupted; this was surprising in view of the heavy steam-sterilization which the blocks had received initially.

Using the above information as a basis, an experiment was undertaken to find a method of inoculation which would ensure a high percentage of infection. Beech branches with the bark left intact, with the bark entirely removed, or with a strip of bark ½ to 1 in. in width removed from one side were prepared as outlined earlier. A 20-year-old pine plantation growing on alkaline (pH 7.0–7.3) soil was used for the experiment. Roots 1–3 cm in diameter and growing at a depth of 15–20 cm were exposed, cleaned, and inoculated as described. Inoculum blocks were held in position with wire or strong cord.

Roots received the following treatments:

(*a*) De-barked blocks were placed against the roots and the inoculation site covered with polythene.

(*b*) Blocks with a strip of bark removed were placed with the exposed peripheral wood touching the pine roots; inoculation sites were covered with polythene.

(*c*) One end of the inoculum was placed against the roots, and the inoculation sites were covered with polythene.

(*d*) As in (*c*) but with no polythene covering.

(*e*) Roots were injured to expose the inner bark and wood; one end of the inoculum was placed against the injury, and inoculation sites were covered with polythene.

All roots were then re-covered to their former depth with the original soil.

The maximum extent of fungal invasion in roots subjected to the foregoing treatments may be seen in Table I. All methods gave satisfactory results, with more than one-half of the roots showing a well-established infection after the relatively short period of 7 months. With the exception of treatment (*a*), approximately one-fifth of the roots had succumbed to the disease. The mean maximum extent of mycelial growth away from the point of inoculation was 26 cm in roots which had died during the study period and only 3 cm in roots

TABLE I

Number of *Fomes annosus* infections and maximum extent of mycelial growth in pine roots 7 months after inoculation with inoculum treated in various ways

Treatment*	Total no.	Number infected	Maximum extent of fungus growth from inoculum (cm)			
			0†	1–5	6–10	>10
a	25	15	5	6	2	2(1)‡
b	20	11	5	2	0	4(4)
c	22	15	5	5	0	5(5)
d	12	11	1	6	2	2
e	40	25	4	11	2(1)	8(8)

*See text for details of treatments.
†Fungus present in the root but growth not extending beyond the site of inoculation.
‡Numbers in parentheses refer to the total number of dead or dying roots in each category.

which had remained alive. Injury to roots before inoculation had no apparent influence on the number of infections or on growth of the fungus. The highest proportion of infections (11 out of 12) occurred where the cut end of the beech blocks were placed against the root, the inoculation site not having been covered with polythene (treatment *d*). Although the sample was small it would appear that a protective covering over the inoculation site is not necessary for successful infection, at least in the wetter winter months. A high proportion of blocks covered with polythene bore a superficial growth of other fungi, especially *Penicillium* and *Trichoderma* spp., when removed for final inspection. It is recommended that if treatment (*d*) is employed in future studies, the peripheral bark of blocks should be retained as a deterrent to rapid replacement of *F. annosus* by organisms entering from the soil. Care must be taken to place the exposed wood of the blocks against the roots since *F. annosus* mycelium is apparently unable to penetrate beech bark. Strong cord satisfactorily maintained the inoculum in position and was less damaging to the roots than wire.

Extent of fungal growth following inoculation of healthy roots is recorded in Table II. Nearly one-half of the roots of suppressed trees had died during the course of the experiment while only one out of nine roots of dominant trees had succumbed. More than one-half of the roots of suppressed trees had infection extending more than 5 cm from the inoculation site while only one

TABLE II

Maximum extent of *Fomes annosus* growth in roots of suppressed and dominant pine trees 7 months after inoculation

Tree class	Total no.	Maximum extent of fungus growth from inoculum (cm)			
		0*	1–5	6–10	>10
Supp.	32	4	10	2	16†
Dom.	45	13	23	3	6‡

*Fungus present in the root but growth not extending beyond the site of inoculation.
†Fifteen out of 16 roots dead or dying as a result of *F. annosus* infection.
‡Five out of six roots dead or dying as a result of *F. annosus* infection.

out of five roots of dominant trees fell into this category. No correlation was found between growth of the fungus and root diameter under the conditions of this experiment.

Mycelium was confined to the inner bark and peripheral woody tissues in many of the living roots examined. Resin impregnation of the wood as a result of fungal invasion occurred where the mycelium was well established. The extent of epiphytic mycelial growth on the living roots varied considerably but in the majority of cases it preceded invasion of deeper tissues by 1–5 cm. The fungus was present throughout the tissues of roots which had died during the course of the study; the extent of growth in the wood was equal to, or well in advance of, that on the outer bark scales.

The food-base requirements of *Fomes annosus* were investigated by using beech inoculum of various weights. Branches 2–4 cm in diameter were cut into blocks weighing 100, 30, 10, and 2–5 g, sterilized in an autoclave, and inoculated with a sawdust culture of *F. annosus*. The 100- and 30-g blocks were incubated at 22° C for 16 weeks and the 10- and 2- to 5-g blocks for 12 weeks. After incubation the fungus was well established in the wood but no advanced decay was present. Unfortunately, a period of only 7 months (September–March) was available for this work. Roots of 20-year-old dominant pine trees, growing on alkaline (pH 7.0–7.5) soil, were inoculated as in treatment (*c*). The roots selected were 1–2 cm in diameter and lay at a depth of 15–25 cm.

The highest proportion of infections occurred when inoculum blocks weighing 10 g (9 out of 10) and 30 g (7 out of 10) were used as the food base, one-third of the roots in the former having died from root rot (Table III). In this experiment only one-half of the roots inoculated with 100-g inoculum became infected. When a similar inoculum was used in a study of inoculation techniques, as outlined earlier, 7 out of 10 roots showed fungal invasion. That beech blocks 2–5 g in weight contained enough food reserve for *F. annosus* to penetrate the bark of healthy pine roots and become established in the wood was evident from the presence of infection in 4 out of 10 roots. Susceptibility of *F. annosus* to replacement in the small 10- and 2- to 5-g blocks was evident from the fact that 17 out of 21 contained only other organisms at the end of the experiment.

TABLE III

Number of Scots pine roots infected with *Fomes annosus* and mean maximum growth of the fungus 7 months after inoculation with inoculum of various weights

Inoc. wt. (g)	Total no.	Number infected	State of infected roots			
			Living		Dead	
			No.	Mean max. growth (mm)	No.	Mean max. growth (mm)
100	10	5	4	10	1	320
30	10	7	7	16	0	—
10	11	9	5	10	4	290
2–5	10	4	4	11	0	—

Beech-branch inoculum was also used to investigate the growth of *F. annosus* in attached and distal portions of severed roots *in situ*. Roots of 20-year-old pine in alkaline (pH 7.0–7.5) and acid (pH 3.8–4.3) soils, lying at a depth of 15–25 cm, were exposed and brushed free of soil. At approximately 70–90 cm from the base of the tree a piece 8–12 cm long was cut from each root with a saw cleaned with methylated spirits. Inoculum, prepared as outlined earlier, was forced between the cut ends of the roots. This procedure was designed to produce infection of the distal portions of the severed roots as well as the portions still attached to the tree, the latter being referred to hereafter as attached roots. Sterile beech blocks replaced infected blocks in the controls. Polythene was wrapped around the site of inoculations and soil was replaced around the roots to its original depth. The number of infections and the extent of fungal growth are presented in Tables IV and V.

TABLE IV

Number of infections and mean maximum extent of growth of *Fomes annosus* 12 months after inoculation of the proximal portions of severed pine roots in alkaline and acid soils

Tree class	Total no.	Number infected	Mean maximum extent of growth of the fungus from inoculum (cm)		
			Outer bark scales	Living bark	Wood
		Alkaline soil			
Suppressed	12	11	17	19	15
Dom., interior	7	7	20	15	1
Dom., marginal	7	6	10	11	0.4
		Acid soil			
Suppressed	8	4	13	20	11
Dom., interior	7	2	1	4	5
Dom., marginal	7	5	9	14	1

TABLE V

Number of infections and mean maximum extent of growth of *Fomes annosus* 12 months after inoculation of the distal portions of severed pine roots in alkaline and acid soils

Tree class	Total no.	Number infected	Mean maximum extent of growth of the fungus from inoculum (cm)	
			Outer bark scales	Wood
		Alkaline soil		
Suppressed	12	11	29	67
Dom., interior	7	7	68	85
Dom., marginal	7	6	44	64
		Acid soil		
Suppressed	8	4	15	91
Dom., interior	7	4	8	53
Dom., marginal	7	6	22	42

Considerable variation in growth was apparent in each category. No difference in the extent of fungal growth was evident between attached roots located in alkaline and acid soils under the conditions of this experiment. Survival of the fungus in inoculum blocks was much reduced in acid soils (50%) as compared with that in alkaline (90%). In all roots of dominant marginal[3] trees mycelial growth was less than in roots of suppressed trees; the difference, however, was large only for growth in the wood. Little variation was observed in the extent of mycelial growth in the inner bark and wood of attached roots of dominant marginal and dominant interior[4] trees.

Severing the roots resulted in a heavy resin impregnation of the wood, extending, in some instances, from the inoculation site as far as 15–20 cm up the attached root. Mycelium had failed to penetrate this region during the 12-month test period. In several instances, however, *F. annosus* had successfully bypassed the central impregnated region by rapid growth in the living bark and peripheral tissues, and had invaded the normal wood beyond. Some resin production resulting from fungus activity was evident in living roots where disease was well advanced.

Fomes annosus had penetrated up to 90 cm in the distal severed roots (Table IV). In the majority of roots, growth in the wood progressed well in advance of that in the outer bark scales. The mean maximum extent of epiphytic growth on dead roots in acid soils was significantly less than that in alkaline soils under the conditions of this experiment.

No infection had taken place in the 12 control plants sampled in this study.

Discussion

Attempts to inoculate the surface of pine roots with *F. annosus* met with failure in the first instance. However, modifications of the initial technique resulted in a satisfactory proportion of infections in roots growing in alkaline soils.

The following prerequisites appear to be necessary for ensuring *F. annosus* infection of pine roots by artificial means: the fungus must be able to move rapidly out of the substrate placed in contact with the roots; the fungus must be growing in a substrate which retards rapid replacement by other organisms or conversely, the inoculation site must be so treated as to minimize the number of organisms capable of replacing *F. annosus* in the inoculum block. When beech inoculum was placed so that the fungus was in direct contact with pine roots, blocks weighing only 2–5 g contained sufficient food reserve for *F. annosus* mycelium to penetrate the bark of the roots and infect the wood beyond; this happened even though replacement of *F. annosus* in some blocks was complete in the 7-month test period. When using beech inoculum care must be taken to place the cut end of the block against the root because *F. annosus* is apparently unable to penetrate the beech bark.

When growth of *F. annosus* was studied in trees of contrasting vigor (suppressed, dominant interior, and dominant marginal), the mean extent of mycelial growth in suppressed trees exceeded that in dominant. The difference

[3]Marginal = trees on the margin of a plantation.
[4]Interior = trees well within a plantation.

in growth of the fungus between categories was less pronounced in the attached portions of severed roots, as would be expected, since severing the roots undoubtedly reduced host vigor as a factor in retarding fungus invasion. Where a root had died during the experiment or where infection had occurred in roots severed from the tree, no consistent difference in fungus development between tree vigor classes could be detected.

Rishbeth (7) suggests that resin may retard or check the growth of *F. annosus* in pine roots. The present investigation provided further evidence on the effectiveness of resin in retarding growth; the fungus failed to invade the central heavily resin-impregnated tissues of the attached roots except in a few instances where, by rapid growth in the living bark and peripheral wood, it had successfully bypassed this region and had invaded the normal wood beyond.

Replacement of *F. annosus* by other organisms in inoculum blocks occurred more frequently in acid than in alkaline environments. These results are in accordance with those of Rishbeth (6), who found that *F. annosus* was rapidly replaced in roots and stumps of pine in acid soils of East Anglian plantations. This he attributed to the higher population of competing organisms, such as *Trichoderma* and *Penicillium* spp., found under these conditions.

Spore Infection

Infection of living Scots pine roots by basidiospores of *Fomes annosus* was probably first postulated by Hartig (3) in 1878. More recently the presence of viable spores of *F. annosus* in forest soil has been demonstrated by Rishbeth (6, 7). Rishbeth failed to obtain mycelial growth from spores inoculated into unsterilized soil; inoculation experiments suggested that living roots probably could not be infected by spores. Molin (4), having successfully washed spores through a column of sand, postulated that localized infections in the field which could not be traced to decayed wood might have arisen as a result of spore infection. Braun (1) suggested that *F. annosus* infection takes place through root contact with infected roots or by spores that are washed into the soil.

The possibility that the rhizosphere of living pine roots might serve as a suitable habitat for germination and development of *F. annosus* spores required clarification. Accordingly, experiments were undertaken to determine the ability of spores to infect pine roots in the laboratory and *in situ*. A technique was developed by which it was possible to examine spores on the surface of bark, where they might be expected to occur naturally.

Root Infection

Spore infection of severed pine roots was studied in the laboratory. Fresh roots of diameters 2, 5–7, and 10–12 cm were cut into 10-cm lengths. Cut ends were sealed with wax to reduce moisture loss and to prevent direct infection of the wood. Roots were treated as follows:

(*a*) lightly brushed to remove adhering soil and washed with distilled water;
(*b*) bark surface sterilized with methyl alcohol;
(*c*) dead bark scales peeled from the roots;
(*d*) roots cut to expose inner bark and woody tissues.

A spore suspension containing 1×10^4 *F. annosus* basidiospores per ml was sprayed on the root surface with an atomizer. Inoculated roots were inserted into sterile, plugged tubes containing moist filter paper, the tubes in turn being placed in polythene bags to restrict moisture loss. At the start of the experiment, replicate samples were inoculated at 10, 15, and 22° C for 10 days. It soon became apparent, however, that temperature was having little effect on germination, as recorded by the presence of conidia, so later tests were conducted at 15° C only. After incubation the roots were split and re-incubated at 22° C for 3 days to allow the characteristic conidia to develop. Root size had no apparent effect on spore germination so the data have been grouped for convenience (Table VI).

TABLE VI

Infection in the laboratory following inoculation with *Fomes annosus* spores of pine roots removed from alkaline and acid soils

	Treatment							
	Cleaned		Surface-sterilized		Loose bark scales removed		Injured	
Site	Total no.	No. inf.	Total no.	No. inf.	Total no.	No. inf.	Total no.	No. inf.
Alkaline	125	1	18	10	20	1	82	68
Acid	60	0	6	6	20	0	40	33

Infection, as recorded by the presence of conidia, failed to occur in all but 2 out of 225 unsterilized roots where bark had remained intact (treatments *a* and *c*). Germination occurred on 80% of the injured root lengths, growth of mycelium being somewhat more profuse where a thin layer of inner bark overlaid the wood. Mycelium had successfully penetrated the bark and invaded the wood of most surface-sterilized roots.

Spore infection of living roots was investigated in dominant and suppressed trees in 20-year-old pine plantations on alkaline (pH 7.3) and acid (pH 4.2) sites. Roots of two sizes, less than 0.5 cm and 1.0–2.0 cm in diameter, lying at depths of 15–20 cm and 50–60 cm, were exposed and treated at approximately 60 cm from the tree base as follows:

(*a*) lightly brushed to remove adhering soil;
(*b*) surface-sterilized by light rubbing with methyl alcohol and, following inoculation, covered with sterile polythene;
(*c*) cut to expose inner bark and woody tissues;
(*d*) cut to expose woody tissues, then severed 20 cm from the tree base.

A suspension containing 1×10^4 spores per ml was sprayed onto the treated roots with an atomizer, and the original soil replaced around the site of the inoculation. Sterile distilled water replaced the suspension in the controls. Roots were sampled after 10 weeks and after 12 months, at which time they were removed to the laboratory, split, incubated for 3 days, and examined for the presence of conidia.

Root size, depth of inoculation, and type of site had no apparent effect on

TABLE VII

Infection of pine roots in a 20-year-old plantation 10 weeks and 12 months after inoculation with *Fomes annosus* spores

	Treatment							
	Cleaned		Surface-sterilized		Injured		Severed	
Infection period	Total no.	No. inf.	Total no.	No. inf.	Total no.	No. inf.	Total no.	No. inf.
10 weeks	14	0	5	0	24	14	8	2
12 months	28	0	12	0	36	9	10	2

development of mycelium so the results have been grouped for convenience (Table VII). No infection took place where roots remained intact. A heavy growth of contaminating organisms was present on many surface-sterilized roots, possibly accounting for the failure of *F. annosus* to grow under these conditions. Spore germination on injured roots, as evidenced by conidial development, had occurred on 14 out of 24 and 9 out of 36 samples after 10 weeks and 12 months respectively. After 10 weeks, mycelium was present on the surface of the wound only, no invasion of the wood being evident. After 12 months, seven out of the nine infected roots had only a small patch of conidia on the wound surface, while in the remaining two the fungus had penetrated the wood to a distance of about 6 cm from the inoculation.

In view of the number of infections in injured roots still attached to the tree, the low percentage take of *F. annosus* in severed roots was surprising. Many of the latter did, however, contain a high population of contaminating organisms, especially blue stains, which may have prevented growth of *F. annosus*. In the two severed roots infected after 10 weeks, mycelium was confined to wood 2–3 mm below the wound; in the two infected after 12 months the fungus was found 15–17 cm from the inoculation point.

Some Factors Affecting Germination of Spores and Growth of Mycelium

From the results of the foregoing field and laboratory studies it was evident that *F. annosus* spores were not able to infect uninjured pine roots. It was felt, however, that a technique was required which would permit a more direct appraisal of spore development on the surface of roots *in situ* as well as in the laboratory. In laboratory studies roots 0.5–1.0 cm in diameter were cut into 5-cm lengths and the loose outer bark scales which showed signs of decomposition were removed. A heavy spore suspension contained on a wire loop was rubbed in a thin film over a small patch on the roots; the small amount of excess moisture in this film was slowly absorbed by the bark so that the spores closely adhered to the surface. Root lengths, except those to be covered with soil, were then placed in sterile moist chambers which, in turn, were put into polythene bags to reduce moisture loss. These were incubated at 15° C for periods as stated in each study. Each moisture chamber consisted of a 6 × 2 cm petri dish with moist filter paper in the bottom and top, the paper being fixed to the lid with solidified agar. When it was desired

to test germination of spores in the presence of soil, root lengths contained in 6 × 2 cm petri dishes were covered with moist soil which was carefully pressed around the bark. Dishes were then placed in polythene bags and treated as above. After incubation the inoculated bark scales were removed from the roots, cut into 0.5-cm squares, and stained in cotton blue for 15 minutes. Sections were scanned with 2/3 and 1/6 microscope objectives but oil immersion was required to obtain accurate measurements. Twenty fields scattered at random were counted on each root.

In field studies uninjured roots, except where otherwise stated, were prepared and inoculated as in the above laboratory procedure. Where it was desired to observe spore germination on roots in the absence of soil, a polythene covering was placed over, but not touching, the inoculation site. Soil was replaced to its original depth in all instances. Following incubation, roots were severed, placed in test tubes containing moist filter paper, and returned to the laboratory for staining within 3 hours of collecting. Cover slips bearing spores, contained in moist chambers placed in polythene bags, were buried in the soil adjacent to the test roots for comparison.

Distilled water, in which *F. annosus* spores readily germinate, was used as the spore suspension medium when it was desired to test germination and growth in the absence of added nutrients. A 3% malt solution adjusted to pH 4.5 gave satisfactory germination of spores and was used where an added nutrient supply was desired. Roots of 20-year-old pine growing at a depth of 15–25 cm in alkaline arable (pH 7.5) and acid (pH 4.3) soils were used in laboratory experiments. Field trials were confined to roots of the alkaline arable plantation.

Measurement of spores in water suspension incubated at 15° C were made 4 days after inoculation; measurement of spores in 3% malt were made 65 hours after inoculation. Field trials were conducted during February and March when soil temperatures varied between 5–7° C. A 12-day incubation period was required in the field before measurement of growth could be taken.

Germinability of basidiospores in distilled water varied considerably, 6–16%, between sporophores (Table VIII). Germination in 3% malt at 15° C was 3–5 times greater and 24–48 hours in advance of that which occurred in distilled water. When spores in water suspension were added to the surface of roots, a small increase was recorded in percentage germination over that which took place on cover slips. This was to be expected, as a small quantity of accumulated nutrients from decomposition and excretion was undoubtedly available. The nutrient level was, however, not sufficient to increase the rate of germination and growth of mycelium as measured here. Similar results were obtained when roots were inoculated *in situ* (Table IX).

Mycelium was apparent on all roots inoculated with a water suspension of spores but no penetration of the bark was observed. Where wood of the root was inoculated directly, almost complete germination occurred and the mycelium rapidly penetrated throughout the tissues. In the belief, then, that the nutrient status of the root surface was insufficient to support a growth of mycelium which could penetrate the bark, spores were inoculated in a 3% malt solution. Percentage and rate of germination were increased significantly

by the addition of malt; mycelium, however, grew away from the inoculation spot only a short distance and failed to penetrate the bark.

The high incidence of *F. annosus* which occurs on alkaline arable soils might conceivably be attributable, in part, to conditions in these areas which are optimum for spore germination and conducive to mycelial penetration of roots. Growth of the fungus on lengths of roots from alkaline and acid soils was subsequently examined in the laboratory (Table VIII). No significant difference was apparent between the two sites with respect to germination of spores and growth of mycelium on root lengths not covered with soil. When roots were covered with soil from their respective sites, the percentage germination of spores in water suspension was much greater under acid conditions whilst it was little different under alkaline; growth of the mycelium was not significantly altered. These findings are in direct contrast to the above hypothesis.

The possibility that soil microorganisms, by intense competition or by production of antibiotics, may suppress growth of *F. annosus* on root surfaces was examined. Root lengths were steam-sterilized, one group at 15 lb pressure for 15 minutes and another at 5 lb pressure for 5 minutes. These were then inoculated with spores in water suspension and incubated in moist dishes for 4 days at 15° C. Nearly complete germination took place in both groups and extensive mycelial development was evident. Steam-sterilization undoubtedly destroyed most, if not all, of the microorganisms on the root surfaces; however, a release of nutrients resulting from a disruption of bark tissues, evident on most roots, must also have occurred.

In order to minimize tissue disruption and release of nutrients, root lengths were sterilized with propylene oxide. Roots excavated from an alkaline arable site were exposed to propylene oxide fumes for 3 days, after which they were removed to a closet for a further 4 days to allow the fumes to disperse. The roots were then inoculated with a water suspension of spores and incubated in moist dishes for 4 days at 15° C. Where it was desired to know if the fungus had penetrated the bark, sterilized roots were incubated for 3 weeks at 15° C, split, and re-incubated for 3 days at 22° C to allow the characteristic conidia to develop. Approximately 15% of the spores germinated on the root surface but again no penetration of the bark took place. From these results it would seem that soil microorganisms are not the primary factor limiting spore infection of roots.

The germination of spores and growth of mycelium in contact with soil was subsequently investigated. In the laboratory, roots were prepared and covered with soil from the respective sites as outlined earlier. In field trials, care was taken to replace the original soil around the point of inoculation. Spores were incubated at 15° C for 4 days in the laboratory and for 12 days in the field.

Spore germination, although variable, was present on all roots examined, and was notably good where roots had been covered with acid soil in the laboratory (Tables VIII and IX). More significant, however, was mycelial development. Growth had apparently ceased shortly after germination, mycelial length seldom exceeding 20 μ, even when the spores had been applied

TABLE VIII

Percentage germination of *Fomes annosus* spores and extent of mycelial growth on pine roots removed from alkaline and acid soils

Control*			Alkaline soil		Acid soil	
% germ.	Mean length of myc. (μ)	Root treatment	% germ.	Mean length of myc. (μ)	% germ.	Mean length of myc. (μ)
		Spores applied in distilled water†				
10	38	Nil	14	25	17	27
11	43	Covered with moist soil	10	8	27	12
32	30	Propylene oxide	37	24		
		Spores applied in 3% malt‡				
34	66	Nil	57	49	67	59
31	71	Covered with moist soil	17	10	25	17

*Growth on a glass cover slip, untreated throughout.
†Sample of eight roots.
‡Sample of six roots.

TABLE IX

Percentage germination of *Fomes annosus* spores and extent of mycelial growth on pine roots growing in alkaline arable soil

	Control*		Soil removed from roots†		Soil in contact with roots‡	
Spore suspension medium	% germ.	Mean length of myc. (μ)	% germ.	Mean length of myc. (μ)	% germ.	Mean length of myc. (μ)
Dist. water	5	15	10	11	7	5
3% malt	31	34	30	36	12	6

*Growth on a glass cover slip.
†Sample of seven roots.
‡Sample of three roots.

in 3% malt solution. Average extent of mycelial growth after 11 days' incubation at 15° C was no greater than after 4 days.

Development of spores on wood surfaces covered with soil was studied in the laboratory. Resin exudation from wounded living roots made field application of this technique impracticable. Roots from 20-year-old pine growing on an alkaline site were treated as follows: wood was shaved smooth with a razor blade, spores were brushed over the surface to ensure maximum contact and moist soil was pressed down around the roots. Roots were incubated at 15° C for 4 days, after which freehand sections were cut from the surface, stained with cotton blue, and observed under the 1/6 objective of the microscope. Percentage germination was high and mycelium was abundant on the surface of the wood of the 10 roots examined.

Discussion

The consistent failure of spores to infect uninjured roots implies that the spread of *F. annosus* by this means is improbable. Some infection may occur

when spores come in contact with woody tissues, particularly in the absence of soil. When soil is in contact with fresh wood, other organisms, particularly blue stains, often infect the tissues ahead of *F. annosus*.

Failure of spores to infect roots might be attributed to biological or nutritional factors. Biologically the rhizosphere microorganisms might suppress mycelial development by intense competition or by the production of antibiotics. Rao (5) noted susceptibility of *F. annosus* to antibiotics as one factor contributing to its poor saprophytic ability. The growth of the fungus on bark of roots surface-sterilized with methyl alcohol may serve to substantiate this latter theory since this treatment presumably destroyed other organisms present. However, the effect of alcohol on the release of organic substances cannot be overlooked. Dobbs and Hinson (2), being unable to obtain germination of many species of spores when the spores were in contact with soil, suggested the presence therein of a widespread fungistatic factor. That this factor might act against *F. annosus* mycelium on the root surface also requires investigation. The failure of spores to infect through the bark of roots sterilized with propylene oxide indicated that microorganisms, if they do have an effect, are not the primary factor.

Conceivably the outer bark may lack sufficient nutrients to supply the required inoculum potential necessary for germinating spores to penetrate the bark. Growth of *F. annosus* on the inner bark and wood of roots might be correlated with a high concentration of carbohydrates in these tissues. Spores failed to infect roots even when added in 3% malt solution. Further sources of nutrient supply must be examined, however, before it will be possible to conclude whether nutrients are the controlling factor in the penetration of bark by mycelium from spores.

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CHROMOSOME NUMBERS AND TAXONOMIC NOTES ON NORTHERN GRASSES

IV. TRIBE FESTUCEAE: POA AND PUCCINELLIA[1]

WRAY M. BOWDEN

Abstract

Chromosome numbers and voucher specimens are recorded for some Canadian and Alaskan collections and a few mainland American collections of *Poa* and *Puccinellia* which belong to the tribe FESTUCEAE. The somatic chromosome numbers of the following are recorded: (1) *Poa agassizensis*, $2n$ = ca. 56; *P. alpina*, $2n$ = 32, 33, 39, and 42; *P. ampla*, $2n$ = 62; *P. annua*, $2n$ = 28; *P. arctica*, $2n$ = 56, 63, 70, ca. 75, 78, 82, 85, 86, and 106; *P. arida*, $2n$ = ca. 76; *P. canbyi*, $2n$ = 56, 70, and ca. 98; *P. cusickii*, $2n$ = 56, and 59; *P. eminens*, $2n$ = 42; *P. fernaldiana*, $2n$ = ca. 42; *P. glauca*, $2n$ = 42, 50, 56, 64, 65, 70, 75, and 78; *P. glaucantha*, $2n$ = 44; *P. glaucifolia*, $2n$ = 56, ca. 70, and ca. 100; *P. interior*, $2n$ = 28, 34, 42, and 56; *P. juncifolia*, $2n$ = ca. 60; *P. leptocoma*, $2n$ = 42; *P. nervosa*, $2n$ = 56, and ca. 74; *P. palustris*, $2n$ = 28, 30, and 32; *P. pratensis*, $2n$ = 28, 42, 44, 56, ca. 66, 67, 70, 74, ca. 78, ca. 82, 84, 86, 88, and ca. 124; *P. saltuensis*, $2n$ = 28; *P. secunda*, $2n$ = 42, 56, 70, ca. 78, 80, and ca. 90; *P. stenantha*, $2n$ = 42; and *P. trivialis*, $2n$ = 14.

(2) *Puccinellia ambigua*, $2n$ = 56; *P. americana*, $2n$ = ca. 42 and 56; *P. angustata*, $2n$ = 42; *P. borealis*, $2n$ = 42; *P. bruggemannii*, $2n$ = 28; *P. cusickii*, $2n$ = 28; *P. distans*, $2n$ = 42; *P. glabra*, $2n$ = 42 and 56; *P. hauptiana*, $2n$ = 28; *P. laurentiana*, $2n$ = 42 and 56; *P. **longiglumis***, $2n$ = 56; *P. lucida*, $2n$ = 42 and 56; *P. nuttalliana*, $2n$ = 42; *P.* × *phryganodes*, $2n$ = 21; *P. vaginata*, $2n$ = 56; and *P. vahliana*, $2n$ = 14.

Introduction

Bowden (1959, 1960*a*, 1960*b*) reported the chromosome numbers of numerous collections of grasses, mostly from Canada. Bowden (1960*a*) treated collections of the tribe FESTUCEAE and noted that the numerous collections of *Poa* and *Puccinellia* would be considered in a later paper in the series. The present paper, the concluding one of this series, is a report on some Canadian and Alaskan collections and a few mainland American collections of *Poa* and *Puccinellia*. Bowden (1959) described the materials and methods that have been used during these investigations.

Results

The species in each genus are arranged alphabetically so that the data can be easily consulted. For each voucher specimen, data are given on the province, district, or state, the cytogenetic accession number (= Cyt. No.), the geographical locality where the material was originally collected, the collector's name and specimen number, and the somatic ($2n$) chromosome number. In the citation of chromosome numbers, the specimens sent in from the field as live plants are designated as "clones" and the plants grown to

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maturity from seed are designated as "plants". All of the voucher specimens are deposited in the Phanerogamic Herbarium of the Plant Research Institute (DAO).

The identification of specimens of *Poa* and *Puccinellia* is a difficult task even for a specialist. Most of the specimens were sent to J. R. Swallen (Smithsonian Institution, Washington) and he gave definite identifications of some specimens and provisional identifications of others. W. G. Dore (Plant Research Institute, Ottawa) identified some of the specimens, and Th. Sørensen (Copenhagen, Denmark) identified a few of the specimens of *Puccinellia*. It is quite likely that some revisions of the identifications of some of these specimens will need to be made in the future when new monographs are available. For the genus *Poa*, I have checked the final identifications with the key and text for *Poa* in Chase's revision of Hitchcock's manual (1951) with the exception of *Poa agassizensis* and *Poa eminens*. *Poa agassizensis* was recently described by Boivin and Löve (1960) and *Poa eminens* was listed in Fernald's revision of Gray's manual (1950) and in Hultén (1942). There is no single key for all of the species of *Puccinellia* and I have used Chase's revision of Hitchcock's manual (1951), volumes 2 and 10 of Hultén (1942, 1950), Sørensen in Porsild (1955), and the monographs by Fernald and Weatherby (1916), Swallen (1944), and Sørensen (1953).

In *Puccinellia*, most of the species have distinctive chromosome numbers but four species have both hexaploids and octoploids. These latter mixtures of polyploids may prove to be due to misidentifications or there may be several infraspecific taxa in each of these four species. In *Poa*, it has been known for many years that there are a series of polyploids in some species. The present list shows only a few species of *Poa* that have a rather uniform chromosome number within each species, for example, *Poa annua* and *Poa palustris*. In several species, a range of polyploid numbers is reported, for example, *Poa arctica*, *P. glauca*, *P. interior*, *P. pratensis*, and *P. secunda*. A new monograph of the North American species of *Poa* is much needed. When such a monograph is available, it is possible that there would be a better correlation between chromosome number and specific identification. The great range of chromosome number in some species may actually indicate the presence of different infraspecific taxa, either subspecies or varieties, or apomicts, and there may be some inter- and intra-specific hybrids. The main value of the present material of *Poa* and *Puccinellia* is that the specimens used for cytological study have been preserved and are available for future monographic work.

While there is a wide range of chromosome numbers in some species of *Poa*, as presently interpreted, the value of the voucher specimens is that the morphological differences between different polyploids in a complex, whether large or small, can be analyzed and taken into account by the taxonomist. In some instances in a polyploid complex, there is a correlation between chromosome number and geographical distribution, for example, the four different octoploid collections of *Poa arctica* from Baffin Island or the four different decaploid collections of *Poa arctica* from the District of Keewatin.

Collections of *Poa*

(1a) Poa agassizensis Boivin & D. Löve

One collection (paratype) of this species had $2n$ = ca. 56. Boivin and Löve (1960) listed this collection. Other collections reported by them had $2n$ = 41, 42, and 43.

Voucher specimen of paratype: Manitoba: Cyt. No. 1226, 1 mile south of Morden, *W. G. Dore 11435*; $2n$ = ca. 56 det. on one plant.

(1b) Poa alpina L.

Four collections showed the numbers, $2n$ = 32, 33, 39, and 42.

Voucher specimens: Manitoba: Cyt. No. 571, Fort Churchill, *J. M. Gillett s.n., July, 1948*; $2n$ = 32 det. on one clone. Cyt. No. 717, Knife Lake, *J. M. Gillett 2271*; $2n$ = 33 det. on one plant. Alberta: Cyt. No. 1213, 5 miles SW. of Twin Butte, north of Waterton Lakes Park, *W. G. Dore s.n., August 1, 1950*; $2n$ = 39 det. on one plant. Alaska: Cyt. No. 1386, Falls Creek, Mile 229, Richardson Highway, *W. J. Cody 6307*; $2n$ = 42 det. on one plant.

Skalińska (1952) reported a range of somatic chromosome numbers for *P. alpina*, namely $2n$ = 14 to $2n$ = 35. Of 113 plants examined, 24 clones had $2n$ = 33 while the numbers $2n$ = 32, 39, and 42 were not encountered. Löve and Löve (1956) reported $2n$ = 22 to $2n$ = 47 for *P. alpina* from Iceland and listed $2n$ = 14 to $2n$ = 74 for previous literature reports for this species. Löve and Löve (1956) studied 178 Icelandic specimens and found $2n$ = 33 in 112 specimens and $2n$ = 44 in 41 specimens. They found one plant with $2n$ = 39.

(1c) Poa ampla Merr.

One collection had $2n$ = 62.

Voucher specimen: Alberta: Cyt. No. 1209, 6 miles north of Waterton Lakes Park, *W. G. Dore s.n., July 28, 1950*; $2n$ = 62 det. on one plant.

Hartung (1946) reported $2n$ = 62 to $2n$ = ca. 100 for collections of *P. ampla*; and Grun (1955) listed $2n$ = 64 and $2n$ = 96. More than half of Hartung's collections had $2n$ = 63.

(1d) Poa annua L.

Three collections were tetraploid, $2n$ = 28.

Voucher specimens: Newfoundland: Cyt. No. 789, near Stephenville, *I. J. Bassett s.n., June, 1949*; $2n$ = 28 det. on one clone. Labrador: Cyt. No. 1059, Goose Bay, *J. M. Gillett s.n., July, 1950*; $2n$ = 28 det. on one clone. Alaska: Cyt. No. 1365, Seward, Kenai Peninsula, *J. A. Calder 5749*; $2n$ = 28 det. on one plant.

Löve and Löve (1956) listed $2n$ = 28 for Icelandic collections of *P. annua* and listed numerous literature reports of the same count. Hovin (1958) reported $2n$ = 14 in four aberrant clones identified as "amphihaploid *Poa annua*".

(1e) Poa arctica R. Br.

Numerous collections of *P. arctica* were studied and a series of polyploid chromosome numbers were observed. Four accessions were octoploid, $2n$ = 56; four collections were decaploid, $2n$ = 70; two collections had $2n$ = 78; and one collection each had $2n$ = 63, ca. 75, 82, 85, 86, and 106.

Voucher specimens: Franklin District: Cyt. No. 576 and 583, Baffin Island,

Frobisher Bay, *H. A. Senn s.n., July, 1948*; $2n=56$ det. on two clones. Cyt. No. 613 and 718, Baffin Island, Frobisher Bay, *J. A. Calder s.n., August, 1948, and J. A. Calder 2213*; $2n=56$ det. on one clone and one plant. Keewatin District: Cyt. No. 683 and 728, Southampton Island, Coral Harbor, *W. J. Cody 2015 and W. J. Cody 1966*; $2n=70$ det. on two plants. Cyt. No. 1105 and 1106, Chesterfield Inlet, ½ mile SE. of settlement, *D.B.O. Savile & C.T. Watts 1564 and 1565*; $2n=70$ det. on two plants. Cyt. No. 723, Southampton Island, Coral Harbor, *W. J. Cody 1906*; $2n=78$ det. on one plant. Alaska: Cyt. No. 1362, head of Resurrection Bay, Seward, Kenai Peninsula, *J. A. Calder 5734*; $2n=$ca. 78 det. on one plant. Franklin District: Cyt. No. 603, Baffin Island, Frobisher Bay, *J. A. Calder s.n., August, 1948*; $2n=63$ det. on one clone. Alaska: Cyt. No. 1361, about 3 miles north of Seward–Hope–Kenai Road Junction, Kenai Peninsula, *J. A. Calder 5767*; $2n=$ca. 75 det. on one plant. Keewatin District: Cyt. No. 725, Southampton Island, Coral Harbor, Hudson Bay Post, *W. J. Cody 1926*; $2n=82$ det. on one plant. Quebec: Cyt. No. 953, near Hudson Bay Post, Great Whale River, *D.B.O. Savile 642*; $2n=85$ det. on one plant. Keewatin District: Cyt. No. 682, Southampton Island, Coral Harbor, *W. J. Cody 2013*; $2n=86$ det. on one plant. Franklin District: Cyt. No. 596, Baffin Island, Frobisher Bay, *J. A. Calder s.n., August, 1948*; $2n=106$ det. on one clone.

Löve and Löve (1948) summarized the literature reports for *P. arctica* and listed a wide range of somatic chromosome numbers, $2n=38$ to $2n=$ca. 100.

(1f) Poa arida Vasey

One collection had $2n=$ca. 76.

Voucher specimen: Alberta: Cyt. No. 1222, 15 miles N. of Wild Horse, southeast corner of Alberta, *W. G. Dore 11998*; $2n=$ca. 76 det. on one plant.

Hartung (1946) listed $2n=63$, 64, 84, ca. 90 and ca. 103 for this species.

(1g) Poa canbyi (Scribn.) Piper

One collection had $2n=56$; three were $2n=70$; and one accession had $2n=$ca. 98.

Voucher specimens: Alberta: Cyt. No. 1197, 15 miles N. of Wild Horse, SE. corner of Alberta, *W. G. Dore 11999*; $2n=56$ det. on one plant. Cyt. No. 1198, 15 miles N. of Wild Horse, southeast corner of Alberta, *W. G. Dore 12000*; $2n=70$ det. on one plant. Cyt. No. 1204, 15 miles north of Cardston, *W. G. Dore & A. J. Breitung 12259*; $2n=70$ det. on one plant. Cyt. No. 1206, 10 miles south of Cardston, *W. G. Dore 12340*; $2n=70$ det. on one plant. Cyt. No. 1220, Manyberries, *W. G. Dore 12023*; $2n=$ca. 98 det. on one plant.

Hartung (1946) reported $2n=$ca. 72, ca. 82, ca. 83, 84, 85, 93, ca. 94, ca. 90, ca. 99, and 105–106 for collections of *P. canbyi*.

(1h) Poa cusickii Vasey

Two collections had the chromosome numbers, $2n=56$ and 59.

Voucher specimens: Alberta: Cyt. No. 1182; northern edge of Milk River Ridge, 40 miles south of Lethbridge, *W. G. Dore 11760*; $2n=56$ det. on one plant. Cyt. No. 1178, Rocky Mt. Foothills, 7 miles south of Cardston, *W. G. Dore 12195*; $2n=59$ det. on one plant.

Hartung (1946) reported $2n=42$ for one collection of *P. cusickii*. Stebbins and Love (1941) reported $2n=28$ for two collections.

(1i) Poa eminens Presl

Two collections were hexaploid, $2n=42$.

Voucher specimens: Newfoundland: Cyt. No. 1411, head of Harbour, St. Anthony, *D.B.O. Savile & J. Vaillancourt 2889*; $2n=42$ det. on one plant. Labrador: Cyt. No. 1126, Goose Bay, Terrington Basin, *J. M. Gillett & W. I. Findlay 5800*; $2n=42$ det. on one plant.

(1j) Poa fernaldiana Nannf.

One collection was hexaploid, $2n=$ca. 42.

Voucher specimen: New York State: Cyt. No. 1821, Mt. Marcy, *D. Erskine s.n., August 1, 1954*; $2n=$ca. 42 det. on one clone.

(1k) Poa glauca Vahl

Ten collections were hexaploid, $2n=42$; two had $2n=50$; 14 were octoploid, $2n=56$; four had $2n=70$; and one accession each had $2n=$ca. 64, 65, 75, and 78.

Voucher specimens: Mackenzie District: Cyt. No. 888, Yellowknife, *W. J. Cody & J. B. McCanse 3031*; $2n=42$ det. on one clone. Cyt. No. 1123, Little Buffalo River, 32 miles west of Ft. Smith, *W. J. Cody & C. C. Loan 4747*; $2n=42$ det. on one plant. Yukon: Cyt. No. 768, Whitehorse, *J. M. Gillett s.n., June, 1949*; $2n=42$ det. on one clone. Cyt. No. 977, Carcross, *J. M. Gillett 3809*; $2n=42$ det. on one plant. Cyt. No. 978, Minto, *J. M. Gillett 4022*; $2n=42$ det. on one plant. Cyt. No. 981, Tantalus Butte, Carmacks, *J. M. Gillett & D. A. Mitchell 4017*; $2n=42$ det. on one plant. Alaska: Cyt. No. 1384, Delta Junction, Mile 268, Richardson Highway, *W. J. Cody & T. J. M. Webster 5953*; $2n=42$ det. on one plant. Cyt. No. 1387, near Delta Junction, *W. J. Cody & T. J. M. Webster 6034*; $2n=42$ det. on one plant. Cyt. No. 1388, near Delta Junction, *W. J. Cody & T. J. M. Webster 6003*; $2n=42$ det. on one plant. Cyt. No. 1390, Big Delta Camp area, Mile 264, Richardson Highway, *W. J. Cody & T. J. M. Webster 6038*; $2n=42$ det. on one plant. Yukon: Cyt. No. 990, South slope of Moosehide Mountain, Dawson, *J. A. Calder & L. G. Billard 3812*; $2n=50$ det. on one plant. Alaska: Cyt. No. 1391, Big Delta Camp area, Mile 264, Richardson Highway, *W. J. Cody & T. J. M. Webster 6037*; $2n=$ca. 50 det. on one plant. Quebec: Cyt. No. 659, Fort Chimo, *J. A. Calder s.n., August, 1948*; $2n=56$ det. on one clone. Manitoba; Cyt. No. 674, Fort Churchill, *J. M. Gillett s.n., August, 1948*; $2n=56$ det. on two clones. Keewatin District: Cyt. No. 724, Southampton Island, Coral Harbor, *W. J. Cody 1923*; $2n=56$ det. on one plant. Mackenzie District: Cyt. No. 917, Yellowknife, *W. J. Cody & J. B. McCanse 3240*; $2n=56$ det. on one plant. Cyt. No. 1124, West of Ft. Smith, *W. J. Cody 4161*; $2n=56$ det. on one plant. Cyt. No. 1054, Fort Smith, *W. J. Cody & C. C. Loan 3933*; $2n=56$ det. on one clone. Cyt. No. 1875, Fort Simpson, *W. J. Cody & J. M. Matte 8785*; $2n=56$ det. on one clone. Yukon: Cyt. No. 771 and 772, Whitehorse, *J. M. Gillett s.n., June, 1949*; $2n=56$ det. on two clones. Cyt. No. 992, West slope of Moosehide Mountain, Dawson, *J. A. Calder & L. G. Billard 3738*; $2n=56$ det. on one plant. Alaska: Cyt. No. 1363, Hope, Kenai Peninsula,

J. A. Calder 5915; $2n$ = ca. 56 det. on one plant. Cyt. No. 1274 and 1392, near base of Donnelly Dome, Mile 250, Richardson Highway, *W. J. Cody 6296*; $2n = 56$ det. on two plants. Cyt. No. 1393, Big Delta Camp area, Mile 264, Richardson Highway, *W. J. Cody & T. J. M. Webster 6150*; $2n$ = ca. 56 det. on one plant. Cyt. No. 1395, Big Delta Camp area, Mile 264, Richardson Highway, *W. J. Cody & T. J. M. Webster 6157*; $2n$ = ca. 56 det. on one plant. Franklin District: Cyt. No. 574, Baffin Island, Frobisher Bay, *H. A. Senn s.n., July, 1948*; $2n = 70$ det. on one clone. Cyt. No. 599, Baffin Island, Frobisher Bay, *J. A. Calder s.n., August, 1948*; $2n = 70$ det. on one clone. Keewatin District: Cyt. No. 726, Southampton Island, Coral Harbor, South Bay, *W. J. Cody 1957*; $2n = 70$ det. on one plant. Mackenzie District: Cyt. No. 1122, Bank of Slave River, west of Ft. Smith, *W. J. Cody & C. C. Loan 4623*; $2n = 70$ det. on one plant. Cyt. No. 1050, Fort Smith, *W. J. Cody & C. C. Loan 3936*; $2n$ = ca. 64 det. on one clone. Keewatin District: Cyt. No. 727, Southampton Island, Coral Harbor, *W. J. Cody 1961*; $2n = 65$ det. on one plant. Franklin District: Cyt. No. 606, Baffin Island, Frobisher Bay, *J. A. Calder s.n., August*, 1948; $2n = 75$ det. on one clone. Quebec: Cyt. No. 648, Fort Chimo, *J. A. Calder s.n., August, 1948*; $2n = 78$ det. on one clone.

Löve and Löve (1956) reported $2n = 42$, 49, 56, and 63 for Icelandic collections of *P. glauca* and listed a wide range of counts from literature reports for this species, namely $2n = 42$ to $2n = 72$.

(1l) Poa glaucantha Gaud.

One collection had $2n = 44$.

Voucher specimen: Nova Scotia: Cyt. No. 1781, Pigeon Point, Isle au Haute, *W. B. Schofield 3760*; $2n = 44$ det. on one plant.

(1m) Poa glaucifolia Scribn. & Williams

Three collections were octoploid, $2n = 56$; and one collection had $2n$ = ca. 70 and another had $2n$ = ca. 100.

Voucher specimens: Alberta: Cyt. No. 1196, 3 miles NW. of town of Bow Island, *W.G. Dore 11865*; $2n = 56$ det. on one plant. Cyt. No. 1200, Manyberries, *W. G. Dore 12056*; $2n = 56$ det. on one plant. Cyt. No. 1224, 10 miles NW. of Lethbridge, *W. G. Dore 11845*; $2n = 56$ det. on one plant. Saskatchewan: Cyt. No. 1187, Piapot, *W. G. Dore 11579*; $2n$ = ca. 70 det. on one plant. Alberta: Cyt. No. 1189, Peigan Indian Reservation, near Brocket, *W. G. Dore 11645*; $2n$ = ca. 100 det. on one plant.

Hartung (1946) reported $2n = 50$, 50 + fragment, and 56 for this species.

(1n) Poa interior Rydb.

Seven collections were tetraploid, $2n = 28$; one accession had $2n = 34$; three collections were hexaploid, $2n = 42$; and six collections were octoploid, $2n = 56$.

Voucher specimens: Saskatchewan: Cyt. No. 1244, Oxbow, on slopes of Souris River Valley, *W. G. Dore & A. J. Breitung 12584*; $2n = 28$ det. on one plant. Alberta: Cyt. No. 1237, Lethbridge, *W. G. Dore 11612*; $2n = 28$ det. on two plants. Cyt. No. 1238, 4200 ft elevation, Milk River Ridge, 40 miles south of Lethbridge, *W. G. Dore 11795*; $2n = 28$ det. on one plant. Cyt. No. 1239, escarpment above Elkwater Lake, Cypress Hills, *W. G. Dore 11921*; $2n = 28$ det. on one plant. Cyt. No. 1241, bank of Dungarvan Creek, 6 miles

north of entrance to Waterton Lakes National Park, *W. G. Dore 12143*; $2n=28$ det. on two plants. Cyt. No. 1242, western slopes of Milk River Ridge, 12 miles east of Cardston, *W. G. Dore 12391*; $2n=28$ det. on one plant. Cyt. No. 1245, 5 miles SW. of Twin Butte, north of Waterton Lakes National Park, *W. G. Dore s.n., August 1, 1950*; $2n=28$ det. on one plant. Saskatchewan: Cyt. No. 1236, 5 miles east of Forget, *W. G. Dore & A. J. Breitung 11521*; $2n=34$ det. on one plant. Quebec: Gatineau Co.: Cyt. No. 1606, Precambrian escarpment north of Breckenridge, *W. G. Dore & R. J. Moore 14454*; $2n=42$ det. on one clone. Ontario: Leeds Co.: Cyt. No. 1898, 3.5 miles NEE. of Mallorytown, *W. G. Dore 17175*; $2n=42$ det. on one clone. Mackenzie District: Cyt. No. 1130, west of Ft. Smith, *W. J. Cody & C. C. Loan 4186*; $2n=42$ det. on one plant. Alberta: Cyt. No. 1131, seven miles north of Ft. Fitzgerald, *W. J. Cody & C. C. Loan 4697*; $2n=56$ det. on one plant. Cyt. No. 1216, Cardston, *W. G. Dore 12299*; $2n=56$ det. on one plant. Mackenzie District: Cyt. No. 1132, Ft. Smith, *W. J. Cody 4145*; $2n=56$ det. on one plant. Cyt.No. 1133, Bell Rock, 8 miles west of Ft. Smith, *W. J. Cody & C. C. Loan 4372*; $2n=56$ det. on one plant. Cyt. No. 1699, Norman Wells, *W. J. Cody & R. L. Gutteridge 7980*; $2n=56$ det. on one clone. Alaska: Cyt. No. 1364, Moose Pass, Kenai Peninsula, *J. A. Calder 6381*; $2n=$ca. 56 det. on one plant.

Hartung (1946) reported $2n=28$ for two collections of *P. interior* from Wyoming.

(1o) Poa juncifolia Scribn.

One collection had $2n=$ca. 60.

Voucher specimen: Alberta: Cyt. No. 1192, Milk River Ridge, 40 miles south of Lethbridge, *W. G. Dore 11787*; $2n=$ca. 60 det. on one plant.

Hartung (1946) listed $2n=62$, 63–64, 78, and 84 for collections of *P. juncifolia*.

(1p) Poa leptocoma Trin.

One collection was hexaploid, $2n=42$.

Voucher specimen: Alaska: Cyt. No. 1359, about 5 miles north of Seward–Hope–Kenai road junction, Kenai Peninsula, *J. A. Calder 6812*; $2n=42$ det. on one plant.

(1q) Poa nervosa (Hook.) Vasey

Two collections were octoploid, $2n=56$, and one accession had $2n=$ca. 74.

Voucher specimens: Manitoba: Cyt. No. 1225, 10 miles east of Virden, *W. G. Dore 11184*; $2n=56$ det. on one plant. Cyt. No. 1232, 12 miles northeast of Melita, *W. G. Dore 11118*; $2n=56$ det. on one plant. Alberta: Cyt. No. 1234, Waterton Lakes National Park, *W. G. Dore s.n., August 6, 1950*; $2n=$ca. 74 det. on one plant.

Hartung (1946) reported $2n=62$ to $2n=70$ for this species.

(1r) Poa palustris L.

Numerous collections were tetraploid, $2n=28$; one accession had $2n=30$ and one had $2n=32$.

Voucher specimens: Newfoundland: Cyt. No. 790, near Stephenville, *I. J. Bassett s.n., July, 1949*; $2n=28$ det. on one clone. Cyt. No. 822, 824, 831,

840, and 857; Gander, *I. J. Bassett s.n., July, 1949*; $2n=28$ det. on five clones. Prince Edward Island: Prince Co.: Cyt. No. 1553, Wellington, *D. Erskine 1407*; $2n=28$ det. on one plant. Alberta: Cyt. No. 1125, 7 miles north of Ft. Fitzgerald, *W. J. Cody & C. C. Loan 4708*; $2n=28$ det. on one plant. Mackenzie District: Cyt. No. 1074, Salt River, *W. J. Cody & C. C. Loan 4587*; $2n=28$ det. on one clone. Cyt. No. 1087, Salt River, *W. J. Cody & C. C. Loan 4603*; $2n=28$ det. on one clone. Cyt. No. 1869, Fort Simpson, *W. J. Cody & J. M. Matte 8790*; $2n=28$ det. on one clone. Cyt. No. 1630, Norman Wells, *W. J. Cody & R. L. Gutteridge 7510*; $2n=28$ det. on one clone. New York State: Clinton Co.: Cyt. No. 1596, West Chazy, 10 miles south of Canada–U.S. border, *W. G. Dore 14303*; $2n=28$ det. on one clone. Newfoundland: Cyt. No. 828, Gander, *I. J. Bassett s.n., July, 1949*; $2n=30$ det. on one clone. Cyt. No. 854, Gander, *I. J. Bassett s.n., July, 1949*; $2n=32$ det. on one clone.

Hartung (1946) reported $2n=28$ for two collections of *P. palustris* and Löve and Löve (1948) listed $2n=28$, 29, 30, and 42.

(1s) Poa pratensis L.

Numerous collections of this species were examined cytologically. The somatic chromosome numbers had a wide range: $2n=28$, 42, 44, 56, ca. 66, 67, 70, 74, ca. 78, ca. 82, 84, 86, 88, and ca. 124. There were more numerous specimens with $2n=56$ and $2n=70$.

Voucher specimens: Newfoundland: Cyt. No. 833, Gander, *I. J. Bassett s.n., July, 1949*; $2n=28$ det. on one clone. Cyt. No. 786, near Stephenville, *I. J. Bassett s.n., June, 1949*; $2n=42$ det. on one clone. Alaska: Cyt. No. 1382, Delta Junction, Mile 268, Richardson Highway, *W. J. Cody & T. J. M. Webster 5938*; $2n=42$ det. on one plant. Labrador: Cyt. No. 1060, Goose Bay, *J. M. Gillett s.n., July, 1950*; $2n=44$ det. on one clone. Cyt. No. 1062 and 1063, Goose Bay, *J. M. Gillett s.n., July, 1950*; $2n=56$ det. on two clones. Cyt. No. 1128, Goose Bay, *J. M. Gillett & W. I. Findlay 5586*; $2n=56$ det. on one plant. Manitoba: Cyt. No. 1816, District of Springfield: Pine Ridge, *B. Boivin, D. Löve, and D. Dunbar 10279*; $2n=56$ det. on one clone. Alberta: Cyt. No. 1227, along the Milk River, Del Bonita District, *W. G. Dore 11808*; $2n=56$ det. on one plant. Alaska: Cyt. No. 1360, Resurrection Creek, Hope, Kenai Peninsula, *J. A. Calder 5927*; $2n=$ca. 56 det. on one plant. Cyt. No. 1385, Falls Creek, Mile 229, Richardson Highway, *W. J. Cody 6327*; $2n=$ca. 56 det. on one plant. Alberta: Cyt. No. 1229, 10 miles SW. of Pincher Creek, *W. G. Dore 12470*; $2n=$ca. 66 det. on one plant. Manitoba: Cyt. No. 565 and 572, Fort Churchill, *J. M. Gillett s.n., July, 1948*; $2n=67$ det. on two clones. Labrador: Cyt. No. 1127, North West River, *J. M. Gillett & W. I. Findlay 5531*; $2n=$ca. 70 det. on one plant. Manitoba: Cyt. No. 1219, North of Windygates and 15 miles SW. of Morden, *W. G. Dore 11332*; $2n=70$ det. on one plant. Cyt. No. 1231, 5 miles north of Rapid City, *W. G. Dore 11211*; $2n=70$ det. on one plant. Alberta: Cyt. No. 1223, near northern edge of Milk River Ridge, 4200 ft elevation, 40 miles south of Lethbridge, *W. G. Dore 11733*; $2n=$ca. 70 det. on one plant. Mackenzie District: Cyt. No. 1129, Ft. Smith, *W. J. Cody & C. C. Loan 4354*; $2n=70$ det. on one plant. Franklin District: Cyt. No. 604 and 608, Baffin Island, Frobisher Bay, *J. A. Calder s.n., August, 1948*; $2n=70$ det. on two clones. Alaska: Cyt. No. 1273 and 1277,

Big Delta Camp area, Mile 264, Richardson Highway, *W. J. Cody 6261 and W. J. Cody 6265*; $2n$=ca. 70 det. on two plants. Cyt. No. 1389, Big Delta Camp area, Mile 264, Richardson Highway, *W. J. Cody & T. J. M. Webster 6045*; $2n$=ca. 70 det. on one plant. Newfoundland: Cyt. No. 788, near Stephenville, *I. J. Bassett s.n., June, 1949*; $2n$=74 det. on one clone. Cyt. No. 1407, St. Anthony, *D. B. O. Savile & J. Vaillancourt 2612*; $2n$=ca. 74 det. on one plant. Franklin District: Cyt. No. 609, Baffin Island, Frobisher Bay, *J. A. Calder s.n., August, 1948*; $2n$=74 det. on one plant. Mackenzie District: Cyt. No. 1049, Fort Smith, *W. J. Cody & C. C. Loan 3935*; $2n$=ca. 78 det. on one clone. Cyt. No. 1256, Lower Hay River, *W. H. Lewis 10*; $2n$=ca. 78 det. on one clone. Prince Edward Island: Queens Co.: Cyt. No. 1501, Bonshaw, *D. Erskine & W. G. Dore 1119*; $2n$=ca. 82 det. on one clone. Cyt. No. 1558, North Rustico, gulf shore, *D. Erskine 1275*; $2n$=ca. 82 det. on one plant. Quebec: Cyt. No. 666, Fort Chimo, *J. A. Calder s.n., August, 1948*; $2n$=84 det. on one clone. Newfoundland: Cyt. No. 877, St. John's, *I. J. Bassett s.n., July, 1949*; $2n$=ca. 86 det. on one clone. Alberta: Cyt. No. 1233, 5 miles SW. of Twin Butte, north of Waterton Lakes Park, *W. G. Dore s.n., August 1, 1950*; $2n$=86 det. on one plant. Keewatin District: Cyt. No. 680, Southampton Island, Coral Harbor, *W. J. Cody 2021*; $2n$=86 det. on one plant. Saskatchewan: Cyt. No. 1230, Hitchcock in SE. Sask., *W. G. Dore & A. J. Breitung 11545*; $2n$=88 det. on one plant. Yukon: Cyt. No. 976, Mayo, *J. M. Gillett & J. A. Calder et al. 4248*; $2n$=88 det. on one plant. Quebec: Cyt. No. 1024: near Hudson Bay Post, Great Whale River, *D. B. O. Savile 641*; $2n$=ca. 124 det. on one plant.

The literature reports for *P. pratensis* show a wide range in somatic chromosome number, for example the numbers listed by Löve and Löve (1956) and Hartung (1946).

(1t) Poa saltuensis Fern. & Wieg.

Two collections were tetraploid, $2n$=28.

Voucher specimens: Nova Scotia: Kings Co.: Cyt. No. 1784, Cape Split, *W. B. Schofield 3305*; $2n$=28 det. on two plants. Quebec: Hull Co.: Cyt. No. 1576, Mackenzie King Estate, Kingsmere, 7 miles NW. of Ottawa, *W. G. Dore 12984*; $2n$=28 det. on one plant.

(1u) Poa secunda Presl

There was a range of somatic chromosome numbers in this species: $2n$=42, 56, 70, ca. 78, 80, and ca. 90.

Voucher specimens: Alberta: Cyt. No. 1188, Purple Springs, 12 miles E. of Taber, *W. G. Dore 11592*; $2n$=42 det. on one plant. Cyt. No. 1199, Manyberries, *W. G. Dore 12032*; $2n$=56 det. on one plant. Cyt. No. 1203, Rocky Mountain Foothills grassland, 7 miles south of Cardston, *W. G. Dore 12194*; $2n$=70 det. on one plant. Cyt. No. 1207, 10 miles SW. of Pincher Creek, *W. G. Dore 12472*; $2n$=ca. 70 det. on one plant. Cyt. No. 1208, Medicine Hat, *W.G.Dore s.n., July 22, 1950*; $2n$=ca. 70 det. on one plant. Cyt. No. 1184, 10 miles NW. of Lethbridge, *W. G. Dore 11849*; $2n$=ca. 78 det. on one plant. Cyt. No. 1211, edge of Red Rock Canyon, Waterton Lakes National Park, *W. G. Dore s.n., July 28, 1950*; $2n$=ca. 78 det. on one plant. Cyt. No. 1173,

Lethbridge, *W. G. Dore 11736*; $2n=80$ det. on one plant. Cyt. No. 1195, 3 miles NW. of town of Bow Island, *W. G. Dore & A. J. Breitung 11856*; $2n=$ca. 90 det. on one plant.

Hartung (1946) reported $2n=$ca. 74 to $2n=$ca. 87 for *P. secunda*. Stebbins and Love (1941) reported $2n=82$, ca. 84, and 86 for this species.

(1v) Poa stenantha Trin.

One collection of this species was hexaploid, $2n=42$.

Voucher specimen: Mackenzie District: Cyt. No. 921, Yellowknife, *W. J. Cody & J. B. McCanse 3489*; $2n=42$ det. on one plant.

(1w) Poa trivialis L.

One collection was diploid, $2n=14$.

Voucher specimen: Nova Scotia: Cape Breton Co.: Cyt. No. 1782, NE. Harbour, Scatari Island, *E. C. Smith et al. 8618*; $2n=14$ det. on four plants.

Löve and Löve (1956) observed $2n=14$ in Icelandic material of *P. trivialis* and cited numerous literature reports that listed the same chromosome number for this species.

Collections of *Puccinellia*

(2a) Puccinellia ambigua Th. Sør.

Two collections were octoploid, $2n=56$.

Voucher specimens: Prince Edward Island: Prince Co.: Cyt. No. 1641, NW. side of Skinner's Pond, 4 miles W. of Tignish, and about 9.5 miles north of the type locality of *P. ambigua* (Alberton), *D. Erskine & A. J. Smith* 2231; $2n=56$ det. on one clone. Cyt. No. 1500, 1 mile southwest of North Point, about 17 miles north of the type locality of *P. ambigua* (Alberton), *D. Erskine & W. G. Dore 1059*; $2n=56$ det. on one clone.

(2b) Puccinellia americana Th. Sør.

Two collections were examined; one was hexaploid, $2n=$ca. 42, and the other was octoploid, $2n=56$.

Voucher specimens: Nova Scotia: Digby Co.: Cyt. No. 1841, St. Bernard, *E. C. Smith et al. 11935*; $2n=$ca. 42 det. on two plants. Prince Edward Island: Queens Co.: Cyt. No. 1494, Bunbury near Charlottetown, *D. Erskine & W. G. Dore 1144*; $2n=56$ det. on one clone.

(2c) Puccinellia angustata (R. Br.) Rand & Redfield

Five collections of this species were hexaploid, $2n=42$.

Voucher specimens: Franklin District: Cyt. No. 1450, 2 miles west of Cape Belknap, northeast of Ellesmere Island, *P. F. Bruggemann 279*; $2n=42$ det. one plant. Cyt. No. 1451, Parr Inlet, northeast of Ellesmere Island, *P. F. Bruggemann 246*; $2n=42$ det. on one plant. Cyt. No. 1540, 1541, and 1542, Mould Bay, Prince Patrick Island, *P. F. Bruggemann 507, 529, and 477*; $2n=42$ det. on three plants.

The hexaploid chromosome number, $2n=42$, was reported for collections of *P. angustata* by Flovik (1940), Holmen (1952), and Jørgensen *et al.* (1958). The latter also reported $n=21$.

(2d) Puccinellia borealis Swallen

Six authentic collections identified by J. R. Swallen were hexaploid, $2n=42$.

Voucher specimens: Saskatchewan: Cyt. No. 1430, Wauchope, Southeastern Saskatchewan, *W. G. Dore & B. Boivin 13181*; $2n=42$ det. on one plant. Cyt. No. 1432, Indian Head, *W. G. Dore & B. Boivin 13057*; $2n=42$ det. on one plant. Mackenzie District: Cyt. No. 1084, Salt Plain west of Ft. Smith, *W. J. Cody & C. C. Loan 4566*; $2n=42$ det. on one clone. Cyt. No. 1747, Norman Wells, *W. J. Cody & R. L. Gutteridge 7821*; $2n=42$ det. on one plant. Yukon: Cyt. No. 979, Selkirk, *J. M. Gillett & D. A. Mitchell 4045*; $2n=42$ det. on one plant. Cyt. No. 1154; Jensen Flats on "The Loop", southeast of Dawson, *J. A. Calder & L. G. Billard 3882B*; $2n=42$ det. on one plant.

(2e) Puccinellia bruggemannii Th. Sør. in Porsild

Chromosome counts were made on seedlings grown from seed from the type collection (*Bruggemann 470*) of this species and two plants were tetraploid, $2n=28$.

Voucher specimens: Franklin District: Cyt. No. 1539, Mould Bay, Prince Patrick Island, *P. F. Bruggemann 470*; grown from loose seed from the type collection, holotype (DAO); specimen grown from seed, greenhouse, March 18, 1953, *W. M. Bowden 1539S2*, hortotype; $2n=28$ det. on two plants.

(2f) Puccinellia cusickii Weath.

Three collections of this species were tetraploid, $2n=28$. This species was included under *P. airoides* in Chase's revision of Hitchcock's manual (1951). J. R. Swallen examined these specimens and considered that they belong to a distinct species. The morphological characteristics are distinctive and the tetraploid count also distinguishes this species from the hexaploid *P. nuttalliana* (the correct name for *P. airoides*).

Voucher specimens: Saskatchewan: Cyt. No. 1142, Piapot, *W. G. Dore 11580*; $2n=28$ det. on one plant. Cyt. No. 1433, Trossachs, 15 miles west of Weyburn, *W. G. Dore & B. Boivin 13241*; $2n=28$ det. on one plant. Alberta: Cyt. No. 1429, Foothills meadow, 7 miles south of Cardston, *W. G. Dore 12187*; $2n=28$ det. on one plant.

(2g) Puccinellia distans (L.) Parl.

Four collections were hexaploid, $2n=42$. These specimens were provisionally identified as *P. distans* by J. R. Swallen; he considered that they are not the same as the European *P. distans* and "seem very close to my *P. borealis*." It seems significant that these four collections and the six collections of *P. borealis* are all hexaploid, $2n=42$. Hubbard (1954) listed $2n=28$ and 42 for the European *P. distans*.

Voucher specimens: Manitoba: Cyt. No. 1148, Winnipeg, *W. G. Dore 11452*; $2n=42$ det. on one plant. Cyt. No. 1150, Lac du Bonnet, *W. G. Dore 12797*; $2n=42$ det. on one plant. Cyt. No. 1151, Winnipeg, *W. G. Dore 11451*; $2n=42$ det. on one plant. Saskatchewan: Cyt. No. 1152, Weyburn, *W. G. Dore 11553*; $2n=42$ det. on one plant.

(2h) Puccinellia glabra Swallen

Two authentic collections identified by J. R. Swallen were examined

cytologically. One specimen was hexaploid, $2n=42$, and the other was octoploid, $2n=56$.

Voucher specimens: Alaska: Cyt. No. 1354, Head of Resurrection Bay, Seward, Kenai Peninsula, *J. A. Calder 6528*; $2n=42$ det. on one plant. Cyt. No. 1353, Homer Spit, Kenai Peninsula, *J. A. Calder 6887*; $2n=56$ det. on one plant.

(2i) Puccinellia hauptiana (Krecz.) Kitagawa

Six collections were tetraploid, $2n=28$.

Voucher specimens: Alberta: Cyt. No. 1117, Ft. Fitzgerald, *W. J. Cody & C. C. Loan 4435*; $2n=28$ det. on one plant. Mackenzie District: Cyt. No. 1870, Fort Simpson, *W. J. Cody & J. M. Matte 8781*; $2n=28$ det. on one clone. Yukon: Cyt. No. 1155, 60 Mile Camp on 60 Mile River near Alaska border, *J. A. Calder & L. G. Billard 4371*; $2n=28$ det. on one plant. Cyt. No. 1156, McRae's Landing on the Stewart River below mouth of McQuesten River, *J. M. Gillett & D. A. Mitchell 4182*; $2n=28$ det. on one plant. Cyt. No. 1158, Mayo, *J. M. Gillett & J. A. Calder et al. 4239*; $2n=28$ det. on one plant. Alaska: Cyt. No. 1396, Shaw Creek, Mile 289, Richardson Highway, *W. J. Cody & T. J. M. Webster 5883*; $2n=28$ det. on one plant.

(2j) Puccinellia laurentiana Fern. & Weath.

Two collections were examined cytologically. One specimen was hexaploid, $2n=42$, and the other was octoploid, $2n=56$.

Voucher specimens: Prince Edward Island: Cyt. No. 1679, Charlottetown, *D. Erskine s.n., August, 1953*; $2n=42$ det. on one clone. Quebec: Bonaventure Co.; Cyt. No. 1446, Tracadigash Point, *I. Ouren & T. Ouren 222*; $2n=56$ det. on one plant.

Church (1949) listed several collections from Quebec and they were octoploid, $2n=56$.

(2k) Puccinellia ***longiglumis*** (Fern. & Weath.) Raymond, comb. nov., based on *Puccinellia paupercula* (Holm) Fern. & Weath. var. *longiglumis* Fern. & Weath., Rhodora, 18:20–21. 1916. Syn.: *Puccinellia paupercula* var. *alaskana sensu* Fern. & Weath., pro parte, Rhodora, 18:18–20. 1916. *Puccinellia pumila* (Vasey) Hitchc. var. *Fernaldii* Hultén, pro parte, Fl. Aleutian Islands, 96. 1937. *nomen invalidum* (no Latin description).

Type locality of *P. longiglumis*: Prince Edward Island: Bunbury. The species ranges from the coast of the northeastern United States to the Maritimes and Eastern Quebec.

J. R. Swallen examined the 11 specimens cited below and he wrote: "[These are] included in the "manual" [Hitchcock, 1951] under *P. pumila*. Fernald includes it under *Puccinellia paupercula* var. *longiglumis*. It does not belong to *P. paupercula*, and is distinct from the western *P. pumila*. Hultén calls it *P. pumila* var. *fernaldii*, but probably is a good species undescribed." Swallen also wrote: "The specimens which I have not named apparently belong to the species which Fernald and Weatherby described under *P. paupercula* and *P. paupercula* var. *alaskana*. Sørensen described *P. ambigua* which was one element in the complex and this is the other one."

I noted a specimen in DAO Herbarium that Marcel Raymond had labelled *Puccinellia longiglumis* and he has given me permission to publish the combination. There is a range of morphological variation in the specimens of *P. longiglumis* as shown by measurements: First glumes, (1.8)–2.1–3.7–(4.1 mm); second glumes, (2.8)–3.2–5.3–(6.5)–(7–9 mm in the type specimen); and lemmas, (3.0)–3.5–4.4–(5.4)–(6.0 mm in the type). The type specimen is an extreme variation with 7–9 mm long second glumes. All of the collections of *P. longiglumis* examined cytologically were octoploid, $2n=56$, including 1 collection from Nova Scotia and 10 collections from the type locality in Prince Edward Island.

Voucher specimens: Nova Scotia: Victoria Co.: Cyt. No. 1471, near mouth of North Aspy River, *E. C. Smith et al. 4270*; $2n=56$ det. on one plant. Prince Edward Island: Queens Co.: Cyt. No. 1488, 1489, 1490, 1491, 1492, 1493, 1495, 1496, 1497, and 1498, Bunbury near Charlottetown, *D. Erskine & W. G. Dore 1145, 1147, 1148, 1149, 1142, 1143, 1146, 1154, 1155, and 1156*, topotypes; $2n=56$ det. on 10 clones.

(2l) Puccinellia lucida Fern. & Weath.

Four collections were examined cytologically. Two specimens were hexaploid, $2n=42$, and two were octoploid, $2n=56$.

Voucher specimens: Mackenzie District: Cyt. No. 1693, Aklavik, Mackenzie River delta, *W. J. Cody & R. L. Gutteridge 7932*; $2n=42$ det. on one clone. Cyt. No. 1695, Aklavik, Mackenzie River delta, *W. J. Cody & R. L. Gutteridge 7931*; $2n=42$ det. on one clone. Quebec: Cyt. No. 1564, Cabbage Willows, James Bay, *G. M. Stirrett 828*; $2n=56$ det. on one plant. Manitoba: Cyt. No. 691, Fort Churchill, *J. M. Gillett 2505*; $2n=56$ det. on one plant.

Church (1949) listed one collection from Quebec and it was octoploid, $2n=56$.

(2m) Puccinellia nuttalliana (Schult.) Hitchc. in Jepson

Seventeen collections of this species were uniformly hexaploid, $2n=42$. In Chase's revision of Hitchcock's manual (1951), this species was listed under *P. airoides* (Nutt.) Wats. and Coult. However, the earliest specific epithet is *nuttalliana* Schult., published in 1824. The significant nomenclature is:

Puccinellia nuttalliana (Schult.) Hitchc. in Jepson, Fl. Calif. 1:162–163. 1912. Based on *Poa nuttalliana* Schult.

Poa airoides Nutt., Gen. Pl. 1:68. 1818. *non Poa airoides* Koel., 1802. *non Poa airoides* Kunth, 1833.

Poa nuttalliana Schult. Mant. 2:303. 1824. Based on *Poa airoides* Nutt.

Puccinellia airoides (Nutt.) Wats. and Coult. in A. Gray, Man. ed. 6. p. 668. 1890. Based on *Poa airoides* Nutt.

Additional later synonyms were listed in Chase's revision of Hitchcock's manual (1951).

The type specimen of this taxon was cited by Nuttall (loc. cit.) as "Hab. in depressed situations around the Mandan [North Dakota] village, on the missouri." The type specimen (PH!; photograph, DAO!) from Mandan, North Dakota, consists of a rather incomplete specimen from which some of the inflorescences have been removed. The type is mounted on a sheet with

several other collections, one of which is labelled as the type of *Glyceria montana* Nutt. ex Buckl. There are also two fragments of the type collection of *Poa airoides*: a panicle (MO!; photograph, DAO!) and a small fragment (US!).

Voucher specimens: Manitoba: Cyt. No. 1116, Gillam, *W. B. Schofield 1329*; $2n=42$ det. on one plant. Saskatchewan: Cyt. No. 1135, Carnduff, southeastern Saskatchewan, *W. G. Dore & A. J. Breitung 12606*; $2n=42$ det. on one plant. Cyt. No. 1139, Weyburn, *W. G. Dore 11552*; $2n=42$ det. on one plant. Cyt. No. 1144, Piapot, *W. G. Dore 11583*; $2n=42$ det. on one plant. Cyt. No. 1149, Chaplin, *W. G. Dore 11564*; $2n=42$ det. on one plant. Alberta: Cyt. No. 1136, 3 miles NW. of Bow Island, *W. G. Dore 11862*; $2n=42$ det. on one plant. Cyt. No. 1137, Taber, *W. G. Dore 11826*; $2n=42$ det. on one plant. Cyt. No. 1138, Medicine Hat, *W. G. Dore 11978*; $2n=42$ det. on one plant. Cyt. No. 1140, 10 miles NW. of Lethbridge, *W. G. Dore 11834*; $2n=42$ det. on one plant. Cyt. No. 1143, Cardston, *W. G. Dore & A. J. Breitung 12298*; $2n=42$ det. on one plant. Cyt. No. 1145, Peigan Indian Reserve, near Brocket, 12 miles east of Pincher Creek, *W. G. Dore 11648*; $2n=42$ det. on one plant. Cyt. No. 1146, shoreline of Elkwater Lake, Cypress Hills, *W. G. Dore 11878*; $2n=42$ det. on one plant. Cyt. No. 1147, Manyberries, *W. G. Dore 12062*; $2n=42$ det. on one plant. Mackenzie District: Cyt. No. 1618, Norman Wells, *W. J. Cody & R. L. Gutteridge 7500*; $2n=42$ det. on one clone. Cyt. No. 1691 and 1743, Aklavik, Mackenzie River delta, *W. J. Cody & R. L. Gutteridge 7930*; $2n=42$ det. on one clone and one plant. Yukon: Cyt. No. 770, Whitehorse, *J. M. Gillett s.n., June, 1949*; $2n=42$ det. on one clone. Cyt. No. 1157, Alaska Highway near Porter Creek, 3 miles north of Whitehorse, *J. M. Gillett 4596*; $2n=42$ det. on one plant.

(2n) Puccinellia × *phryganodes* (Trin.) Scribn. & Merr. (pro sp.)

Three collections were triploid, $2n=21$.

Voucher specimens: Franklin District: Cyt. No. 577, Baffin Island, Frobisher Bay, *H. A. Senn s.n., July, 1948*; $2n=21$ det. on one clone. Cyt. No. 612, Baffin Island, Frobisher Bay, *J. A. Calder s.n., August, 1948*; $2n=21$ det. on one clone. Keewatin District: Cyt. No. 1526, Southampton Island, Coral Harbour, *D. K. Brown s.n., September, 1952*; $2n=21$ det. on one clone.

Sørensen (1953) stated that plants of *P.* × *phryganodes* do not set seed and the anthers do not dehisce nor form pollen. They spread by vegetative reproduction; see also Jørgensen *et al.* (1958). The three specimens cited above have flowering culms. It seems to me that *P.* × *phryganodes* is an interspecific triploid hybrid that has arisen by hybridization between a diploid and a tetraploid species. When more information is available, it might be possible to determine the parentage of this putative hybrid.

The triploid chromosome number, $2n=21$, has been reported for Greenland collections of this taxon by Sørensen (1953) and by Jørgensen *et al.* (1958).

(2o) Puccinellia vaginata (Lange) Fern. & Weath.

One collection was octoploid, $2n=56$.

Voucher specimen: Keewatin District: Cyt. No. 714, Southampton Island, Coral Harbor, South Bay, *W. J. Cody 1523*; $2n=56$ det. on one plant.

Jørgensen *et al.* (1958) reported $2n=56$ for three plants from Greenland.

(2p) Puccinellia vahliana (Liebm.) Scribn. & Merr.

Two collections were diploid, $2n=14$.

Voucher specimens: Keewatin District: Cyt. No. 716, Southampton Island, Coral Harbor, *W. J. Cody 2010*; $2n=14$ det. on two plants. Cyt. No. 730, Southampton Island, Coral Harbor, *W. J. Cody 1975*; $2n=14$ det. on one plant.

Sørensen (1953), Holmen (1952), and Jørgensen *et al.* (1958) listed this taxon as *Colpodium vahlianum* (Liebm.) Nevski. The diploid chromosome number, $2n=14$, was reported for this species by Flovik (1940) and Holmen (1952); and Jørgensen *et al.* (1958) reported $n=7$.

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THE RELATION OF BARK MOISTURE TO THE DEVELOPMENT OF CANKER DISEASES CAUSED BY NATIVE, FACULTATIVE PARASITES

IV. PATHOGENICITY STUDIES OF CRYPTODIAPORTHE SALICELLA (FR.) PETRAK, AND FUSARIUM LATERITIUM NEES., ON POPULUS TRICHOCARPA TORREY AND GRAY, P. 'ROBUSTA', P. TREMULOIDES MICHX., AND SALIX SP.[1]

JOHN E. BIER

Abstract

In the field, *Cryptodiaporthe salicella* has been found only on *Salix* sp., and *Fusarium lateritium* on nursery-grown *Populus trichocarpa* Torrey and Gray. However, the pathogens for these diseases attacked *P. trichocarpa*, *P.* 'robusta', *P. tremuloides* Michx., and *Salix* sp. when inoculations were made on cuttings which were allowed to dry gradually in a dormant condition. The development of the diseases in the field and in the laboratory was related to the moisture content of the host bark. Canker growth occurred only when the relative turgidity of the bark was below 80%. The possibility of employing similar methods of investigation for the discovery and evaluation of canker diseases of importance in disease-testing programs is discussed.

Introduction

In previous studies (1, 2, 3) a close correlation was found to exist between the development of bark cankers caused by facultative parasites and the moisture content of young, living bark. When bark moisture was expressed as a percentage of the amount of water required to saturate the sample under experimental conditions, relative turgidities of 80% or more inhibited canker development, which, however, occurred normally at lower percentages. Season of the year, site conditions, and suppression were shown to be factors which resulted in a reduction of the relative turgidity to levels below the inhibiting value of 80%.

The similarity in behavior of the host–pathogen relationships suggested that facultative parasites as *Cryptodiaporthe salicella* (Fr.) Petrak[2] and *Fusarium lateritium* Nees. may be pathogenic on a number of tree species provided that host vigor is reduced to the level of susceptibility as may be indicated by the relative turgidity of the bark tissues. To test this possibility inoculations of *C. salicella* and *F. lateritium* were made on the following:[3]

Populus trichocarpa Torrey and Gray (black cottonwood, section Tacamaha),

Populus × *Euramericana* (Dode) Guinier cv. 'robusta' (*P.* 'robusta', section Aigeiros),

[1]Manuscript received August 15, 1960.

Contribution from the Department of Biology and Botany, and the Faculty of Forestry (Research Paper No. 32), University of British Columbia, Vancouver 8, B.C. Financial support for this project was granted by the National Research Council of Canada, and MacMillan, Bloedel and Powell River, Limited.

[2]According to Butin (4, 5), *C. salicina* (Fr.) Wehm. should be regarded as a synonym of *C. salicella*.

[3]Classification of the poplars was derived from "Poplars in Forestry and Land Use". FAO Forestry and Forest Product Studies No. 12, Food and Agriculture Organization of the United Nations, Rome, 1958.

P. tremuloides Michx. (quaking aspen, section Leuce),
Salix sp. (probably *S. scouleriana* Barratt).

Methods

The methods of determining the relative turgidity of bark and preparing the burned wound inoculations were the same as those described in the studies on *Cryptodiaporthe* canker (1).

Trial experiments were conducted to determine the effects of storing cuttings with sealed ends, in closed plastic bags that were placed in a cabinet with a temperature ranging from 4° to 7° C. After a 2-month period of storage, microscopic examination of sections from the bark and cambium demonstrated that the cutting stock had remained in an inactive condition without evidence of callus at the margin of wounds. Moisture condensation occurred in the plastic bags and relative turgidity measurements proved a gradual reduction in bark moisture. Further, it was shown that the test fungi continued to grow in plate cultures placed in the cabinet. It was evident, therefore, that the temperature cabinet might serve as a useful incubation chamber for testing the pathogenicity of the fungi on the different hosts.

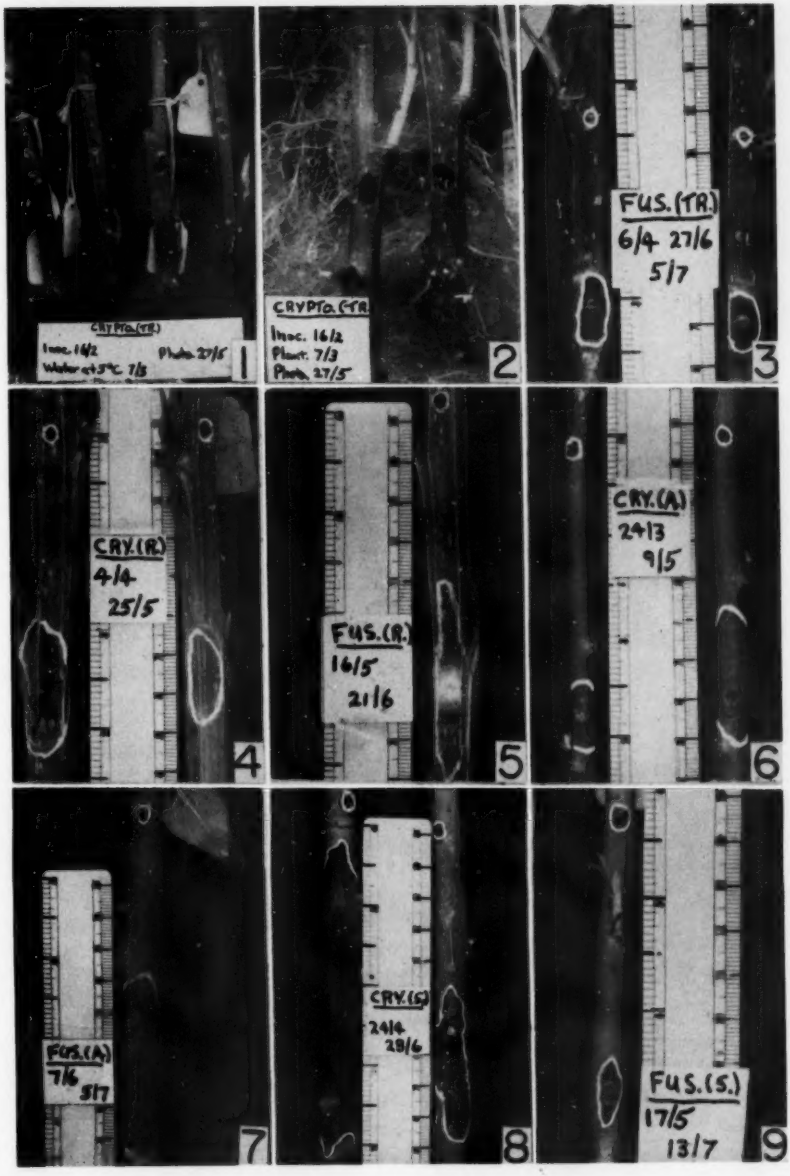
In February, 1960, dormant cuttings were harvested from 1- and 2-year-old shoots of the different trees and measurements were made of their bark moisture content. After the ends were sealed with paraffin, the cuttings were placed in plastic bags that were stored temporarily in the temperature cabinet.

One inoculation and a control were made on each cutting and, after treatment, each sample of cuttings was again placed in the plastic bag and returned to the cabinet for incubation. After canker development had occurred, which was frequently within 1 month after inoculation, a second series of bark moisture measurements was made, and the cuttings planted in vermiculite at laboratory temperature. In several instances conidia of the fungi developed on the cankered areas (Fig. 5), and no difficulty was encountered in reisolating the pathogens from diseased bark. Bark lesions did not develop at the control wounds (Figs. 1 to 9). A third series of bark moisture measurements was made after the planted cuttings had produced shoots and callous tissue at the margin of the lesions (Fig. 3).

Results

The results of the experiments demonstrated that *C. salicella* and *F. lateritium* were causal agents for canker diseases on *P. trichocarpa*, *P.* 'robusta', *P. tremuloides*, and *Salix* sp. when the bark tissues of the hosts were

FIGS. 1 to 9. Upper wounds controls, lower wounds inoculations.
FIG. 1. *Cryptodiaporthe* canker on *P. trichocarpa*, after 20 days of incubation at 5° C.
FIG. 2. As Fig. 1, 81 days after planting in vermiculite.
FIG. 3. *Fusarium* canker on *P. trichocarpa*, after 82 days of incubation at 5° C. Photograph taken 8 days after planting, illustrating callus formation at the margin of the cankers.
FIG. 4. *Cryptodiaporthe* canker on *P.* 'robusta', after 51 days of incubation at 5° C.
FIG. 5. *Fusarium* canker on *P.* 'robusta', after 36 days of incubation at 5° C.
FIG. 6. *Cryptodiaporthe* canker on *P. tremuloides*, after 46 days of incubation at 5° C.
FIG. 7. *Fusarium* canker on *P. tremuloides*, after 28 days of incubation at 5° C.
FIG. 8. *Cryptodiaporthe* canker on *Salix* sp., after 65 days of incubation at 5° C.
FIG. 9. *Fusarium* canker on *Salix* sp., after 57 days of incubation at 5° C.



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conditioned for disease development. Host susceptibility was obtained by gradually drying the bark of dormant cuttings at a temperature of approximately 5° C. Summaries of the inoculation experiments and the bark moisture studies are presented in Table I and in Figs. 1 to 9.

TABLE I

Summary of the inoculation experiments and bark moisture studies on dormant cuttings incubated at approximately 5° C and later planted in vermiculite at laboratory temperature

Tree species	Inoculum	Total no. of inoculations	No. of successful inoculations*	Av. rel. turg., % of bark when harvested	Av. rel. turg., % of bark at time of canker growth	Av. rel. turg., % of bark after planting and shoot growth
P. trichocarpa	*C. salicella*	36	36	80	79	89
	F. lateritium	24	24	82	74	87
P. 'robusta'	*C. salicella*	24	22	75	68	87
	F. lateritium	16	16	76	66	90
P. tremuloides	*C. salicella*	30	24	86	70	92
	F. lateritium	24	24	84	73	89
Salix sp.	*C. salicella*	12	12	78	78	87
	F. lateritium	24	24	77	76	90

*Inoculation failures consisted of diseased cuttings which failed to produce shoots after planting.

From Table I it is evident that disease development for each host–pathogen association occurred when the relative turgidity of the bark was below the 80% level. The relative turgidity values recorded at the time of canker growth are not to be interpreted as threshold levels for disease development. Indeed, the first examination of a number of the inoculation experiments was made after a 2-month absence from the laboratory, and after considerable canker growth had occurred (Figs. 6 and 8). It is believed that earlier investigation of the material would have demonstrated canker growth at higher levels of bark moisture. Further, the bark moisture values recorded at the time of harvesting are not to be considered as averages for the trees during the dormant period. The bark moisture level of a species may vary considerably from week to week depending upon the environmental conditions immediately prior to, and at the time of measurement. This phase of the study will be discussed in a separate contribution dealing with the relation of bark moisture to the rooting capacity of *P. trichocarpa* and *P.* 'robusta'.

Although *P. trichocarpa*, *P. tremuloides*, and *Salix* sp. occur in mixture in the field, *Cryptodiaporthe* canker has been found only on *Salix* sp. Numerous bark moisture measurements made during the past three dormant seasons have shown that *Salix* sp. was the only tree in the stands that consistently had relative turgidity values below 80%, indicating susceptibility to the disease.

Canker growth could be arrested by supplying water to the dormant, infected cuttings in the temperature cabinet. In such instances the relative turgidity of the bark increased to above the 80% level within a period of 48 hours. Indeed, this treatment appeared adequate to retain both the host

and the disease in a more or less static condition for a considerable period of time. This was demonstrated in the inoculation experiment of *C. salicella* on *P. trichocarpa* (Fig. 1). Sixteen dormant cuttings were inoculated on February 16 and allowed to dry gradually in the cold cabinet until March 7, when the cankered areas were as shown in Fig. 1. At this time, eight of the diseased cuttings were supplied with water and returned to the cabinet until May 20, when it was apparent that the material had remained in a dormant condition without further canker growth. The cuttings were then planted in vermiculite in the laboratory and the photograph was taken on May 27, after the initiation of shoot growth (Fig. 1). The remaining eight diseased cuttings were planted in vermiculite on March 7, and all developed vigorous roots and shoots with no further spread of the disease (Fig. 2).

Discussion and Summary

In the Vancouver area *Fusarium* canker has been found only on nursery-grown *P. trichocarpa* (2) and *Cryptodiaporthe* canker on field trees of *Salix* sp. (1). However, by gradually drying cuttings in a dormant condition the pathogens of these diseases attacked *P. trichocarpa*, *P.* 'robusta', and *P. tremuloides* (representative of three sections of the genus *Populus*), and *Salix* sp. Field and laboratory experiments have shown that canker development occurred only when the relative turgidity of the dormant bark was below 80%. As in the earlier studies (1, 2, 3) bark moisture measurements provided useful indices for the level of host resistance to these diseases.

Since *Populus* and *Salix* are closely related genera it is of interest that Mr. B. Sivak, graduate student in forest pathology, employing the methods described in this study, has been successful in obtaining positive inoculations of *C. salicella* on species of *Acer*, *Alnus*, *Cornus*, and *Prunus*. The details of this and other phases of Mr. Sivak's research will be published at a later date. The broad host range demonstrated for *C. salicella* would suggest a limited degree of host specificity for this pathogen and, perhaps, other facultative parasites of a similar type. Investigations into the physiological factors predisposing the hosts to fungous attack would appear to be an important objective in research on the epidemiology of the canker diseases caused by facultative parasites.

The results of this study provide information that may be of interest to those concerned with disease-testing programs. It is evident that the host ranges for some facultative parasites may be greater than is recognized at the present time. For example, although *Cryptodiaporthe* canker has been restricted to *Salix* sp. under field conditions in Vancouver, it may attack species of other genera when the vigor of these hosts is reduced to the level of susceptibility. Within this region or in others where the fungus is known to occur, unfavorable environmental and (or) soil conditions could result in the development of the disease on a number of tree species. The relation between the incidence of disease and site has been demonstrated previously in the studies of *Fusarium* canker on *P. trichocarpa* (2). The recent literature in forest pathology contains many papers which describe "apparently" new canker diseases caused by facultative parasites. The host–pathogen relationships for a number of these diseases may be similar to those demon-

strated for *Cryptodiaporthe* and *Fusarium* cankers.

In the investigation of these diseases it has been necessary to conduct the pathogenicity tests during the dormant period of the hosts when the natural defence reactions of the trees were lessened, or at a minimum. Inoculations made during the period of tree growth have failed to provide an accurate expression of disease potentials. An examination of meteorological records has shown that temperatures favorable to the active growth of the pathogens have occurred frequently, and for lengthy periods, during the dormant seasons of the hosts.

In each of the host–pathogen combinations canker development did not occur until the relative turgidity of the dormant bark was below the 80% level. It is believed that for each host and pathogen it would be possible to establish a relative turgidity value that would serve as the threshold level for disease susceptibility. To accomplish this would require earlier and more frequent bark moisture measurements than were possible in this study. The establishment of these threshold levels for the different hosts of one pathogen may prove of considerable practical importance. For example, in this study there was some evidence that the threshold level for the development of *Cryptodiaporthe* canker may be higher in *P. trichocarpa* than in *P.* 'robusta'.

After the threshold level for susceptibility has been established for a host and pathogen, it may be possible to relate this value to field readings of bark moisture for the tree as it occurs on different sites and in different regions. The assignment of disease vulnerability ratings to different stands and plantations of the host may be feasible. For example, during the dormant period, the trees of some plantations may yield bark moisture values that are consistently below the threshold level. Such plantations would be most vulnerable to the disease. The relation between the incidence of disease in the field and the relative turgidity of the host bark has been demonstrated in this, and in the earlier studies (1, 2, 3).

At present there is considerable interest in organizing an international program for the discovery and evaluation of important foreign tree diseases before they move from one country to another. Investigations on *Populus* sp. may be given primary consideration. An initial objective of the work may consist in the establishment of test plantations in different countries. The information presented in the preceding paragraphs suggests that international co-operation for the screening of some canker diseases caused by facultative parasites may be accomplished in the absence of test plantations. Pathogenicity tests on dormant cutting stock may be adequate for the establishment of threshold levels of bark moisture which indicate susceptibility to some diseases of this type. These threshold levels could be related to the field behavior of the host trees in different countries. Should these methods provide sound results, it is evident that the experimentation could be undertaken very inexpensively, and provide conclusive results within a short period of time.

Preliminary results indicate that the field exposure of dormant cuttings during the non-active period for growth may prove a useful method for the discovery of potential canker diseases caused by facultative parasites.

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THE RELATION OF BARK MOISTURE TO THE DEVELOPMENT OF CANKER DISEASES CAUSED BY NATIVE, FACULTATIVE PARASITES

V. ROOTING BEHAVIOR AND DISEASE VULNERABILITY IN CUTTINGS OF POPULUS TRICHOCARPA TORREY AND GRAY, AND P. 'ROBUSTA'[1]

JOHN E. BIER

Abstract

In cuttings from 1-year-old shoots of *Populus trichocarpa* Torrey and Gray and *P.* 'robusta', correlations were found to exist between the degree of vigor as expressed by the production of basal callus and the associated wound roots, and the relative turgidity of the bark at the time of harvest or planting. The ability to produce basal callus and wound roots decreased in cuttings with relative turgidities below the 80% level, and many were killed by the development of basal cankers caused by *Fusarium lateritium* Nees and *Cytospora chrysosperma* (Pers.) Fr. Therefore, the bark moisture level in cuttings was indicative of their ability to produce basal callus and wound roots in addition to their vulnerability to these canker diseases caused by facultative parasites.

In uniform-sized cuttings of *P.* 'robusta' no relation was found between the production of morphological roots developing from root primordia, and the bark moisture level, or the percentage of plants which survived. Larger numbers of morphological roots developed on cuttings from the basal and, consequently, older parts of stems.

The production of basal callus and wound roots in *P.* 'robusta' was found to be related to the total weight of cuttings of uniform size, which suggested that weight determinations in the field may provide useful guides into the general level of vigor of dormant nursery stock and stored material.

Introduction

Previous studies (2, 3) have shown a correlation between the moisture content of 1-year-old bark of dormant *Populus trichocarpa* Torrey and Gray and *P.* 'robusta',[2] and the development of *Cryptodiaporthe* and *Fusarium* cankers, the pathogens invading the living bark only when the relative turgidity was below the 80% level. It was observed that many cuttings with lower values in bark moisture failed to survive after planting. Examination of the material suggested that lowered vitality evidenced by rapid shoot growth without adequate root growth was the primary cause for mortality (2). These observations indicated the possibility that bark moisture readings taken at the time of harvesting and (or) planting may indicate the rooting performance of cutting stock in addition to its vulnerability to canker diseases caused by facultative parasites.

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Contribution from the Faculty of Forestry (Research Paper No. 34), and the Department of Biology and Botany, University of British Columbia, Vancouver 8, B.C. Financial support for these studies was granted by the National Research Council of Canada, Western Plywoods Limited, and Columbia Cellulose Limited.

[2]In a recent publication of the International Poplar Commission (5) the male hybrid *P.* 'robusta' was described as follows: *Populus* × *Euramericana* (Dode) Guinier cv. 'robusta'.

The Bark Moisture Level, Rooting, and Survival of Cuttings of *P. trichocarpa* Harvested at Different Winter Temperatures

Methods

Bark moisture measurements were made of random samples of the 1958 growth (10 cuttings per sample) harvested in January and February, 1959. The moisture contents of bark were expressed as relative turgidity percentages, using the methods outlined previously (1). After the bark samples were taken the cuttings were placed outdoors with their basal ends in water. The results of previous work (1, 2) had demonstrated this to be a suitable method of storing dormant cuttings. On February 15, when slight bud swelling was observed, all cuttings were planted in clay pots containing sand, which were placed in shallow trays provided with a continuous supply of water. The delay in planting the samples collected in January gave more assurance that all cuttings had completed the period of "physiological" dormancy as described by Richardson (6). Shoot and root counts were made on April 6, when it was apparent that the surviving cuttings would continue to develop into healthy trees.

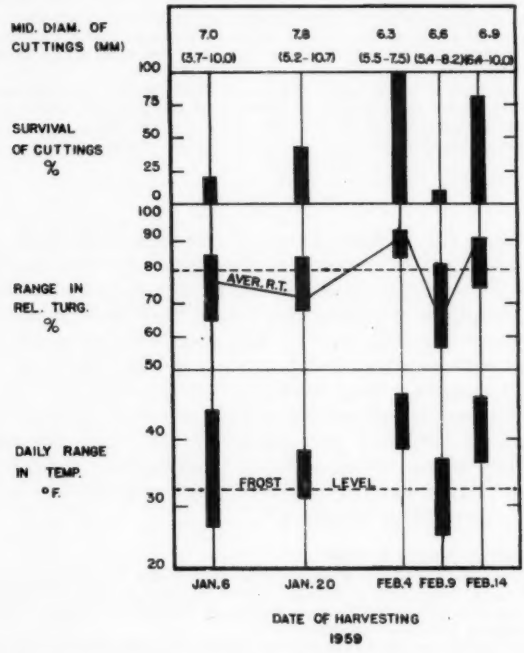

FIG. 1. The bark moisture level and survival of cuttings of *P. trichocarpa* harvested at different winter temperatures.

Results

No apparent relation was found between the average mid-diameter of a sample, and its degree of survival (Fig. 1).

Samples collected on days with below-freezing temperatures had lower values in bark moisture and the average relative turgidity was below 80%. It was felt that the range in bark moisture within a sample was due in part to the moisture gradients which occur in individual shoots, and within the different shoots of one root system (Fig. 1). Numerous bark moisture measurements have shown that during dormancy the relative turgidity percentage was higher in the basal region of individual shoots, and in the largest shoots of one root system.

The average level of bark moisture varied considerably during moderate and cold periods as was evident in the February samples (Fig. 1).

The percentage survival was less in the samples harvested when the average relative turgidity of the bark was below 80% (Fig. 1). Although shoots developed on the cuttings with the lower levels of bark moisture, much of this new growth died prior to April 6, or 56 days after planting (Fig. 2). Examination of the planted portions of the cuttings demonstrated that those with low bark moisture failed to produce a basal callus and the associated wound roots, and became diseased with basal and top cankers caused by *Fusarium lateritium* Nees and *Cytospora chrysosperma* (Pers.) Fr. (Figs. 3, 4). This disease attack was of a secondary nature, spreading from nodal and lenticel infections that developed only in cuttings with the lower values in bark moisture (Fig. 5). When healthy cuttings were placed in a moist chamber,

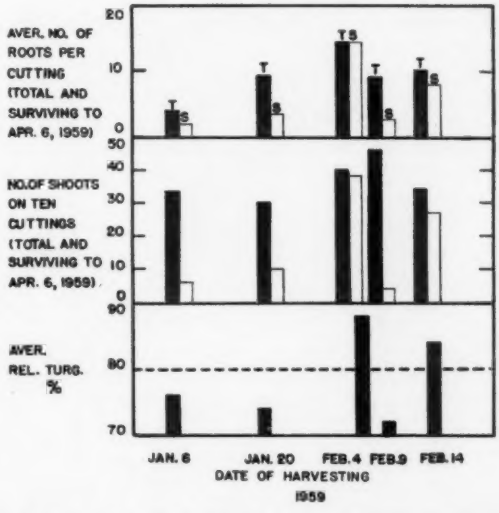

FIG. 2. The relation of the bark moisture level to the growth of cuttings of *P. trichocarpa*.

superficial mycelium of *F. lateritium* and *C. chrysosperma* developed at the base of several of the new shoots, but canker attack did not occur in these instances (Fig. 6).

The Relation of Bark Moisture to the Rooting and Survival of *P.* 'robusta'

The apparent relation of bark moisture with the rooting and with the survival of *P. trichocarpa* indicated the need for further investigations employing more uniform material and methods. *P.* 'robusta' was selected for the studies since this male hybrid was of clonal origin. Further, this tree is of considerable interest locally and internationally, having been widely cultivated in France, Belgium, Germany, and Great Britain.

Method of Harvesting

Cuttings were taken from the larger (major) and the smaller (minor) shoots of the 1959 growth, and were cut to a uniform size, namely, 25 cm long with a mid-diameter of 1 cm. Therefore, shoots with diameters of less than 1 cm were not sampled (Fig. 9). This size of cutting was selected since it conforms with that recommended for propagation purposes by the International Poplar Commission (5). In general, the cuttings of the prescribed size occurred in the upper half of the major shoots, and in the lower half of the minor shoots (Fig. 9). The total weight of each cutting (to 0.1 g accuracy) was determined at the time of harvesting.

Seasonal Variation in Bark Moisture and Total Weight of Uniform-sized Cuttings

At frequent intervals, bark moisture and total weight measurements were obtained for cuttings harvested from the shoots produced in 1959. The classification into major and minor shoots was not made until after complete defoliation had occurred in November. A summary of the results of this work is given in Fig. 10. The cuttings from the major and minor shoots were collected at the same times, and each average value was derived from a sample including a minimum of eight cuttings.

An appreciable reduction in the average values for bark moisture and cutting weight occurred during September. After defoliation, still lower values were obtained which persisted until the latter part of February, 1960 (Fig. 10). During the period from November 15 to February 15, the average total weights and bark moisture levels were consistently higher in the cuttings from the major shoots, which suggested the possibility of a relationship between the total weight of a cutting and its bark moisture content (Fig. 10). After February 15, the average weights of the minor cuttings became greater than that of the major cuttings, although the average bark moisture values remained at lower levels. Since the cuttings from minor shoots were taken

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FIG. 3. Root and shoot growth on the cuttings harvested January 6, when the average relative turgidity of the bark was 76%.

FIG. 4. Root and shoot growth on the cuttings harvested February 4, when the average relative turgidity of the bark was 88%.

PLATE I

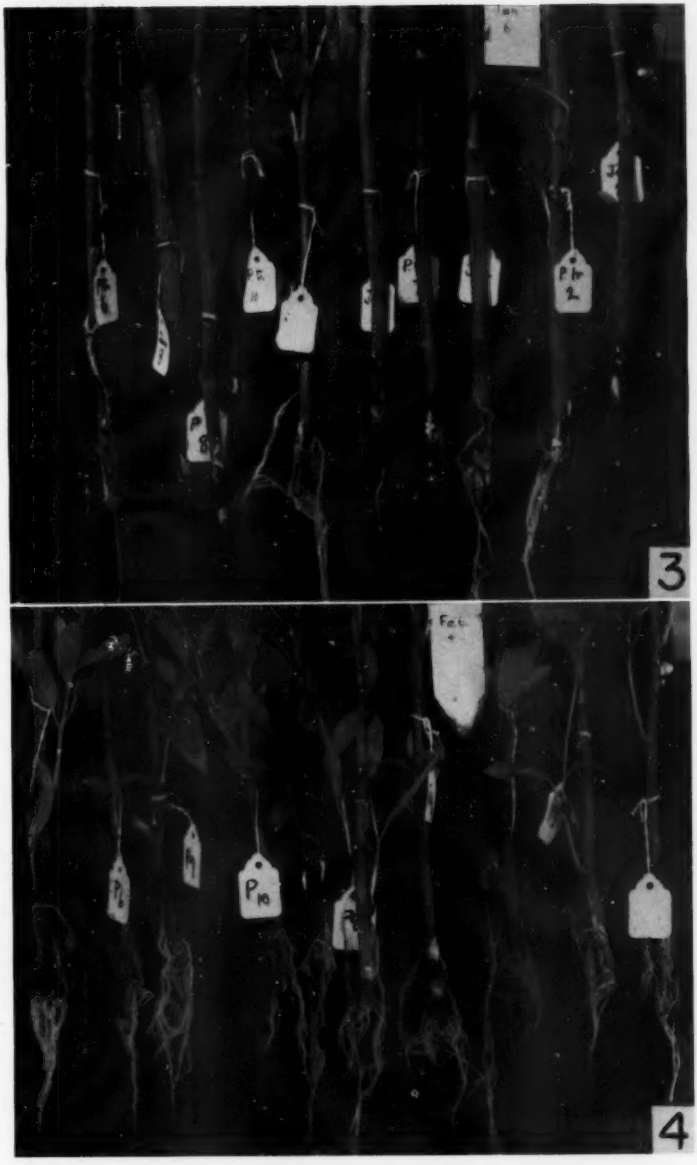

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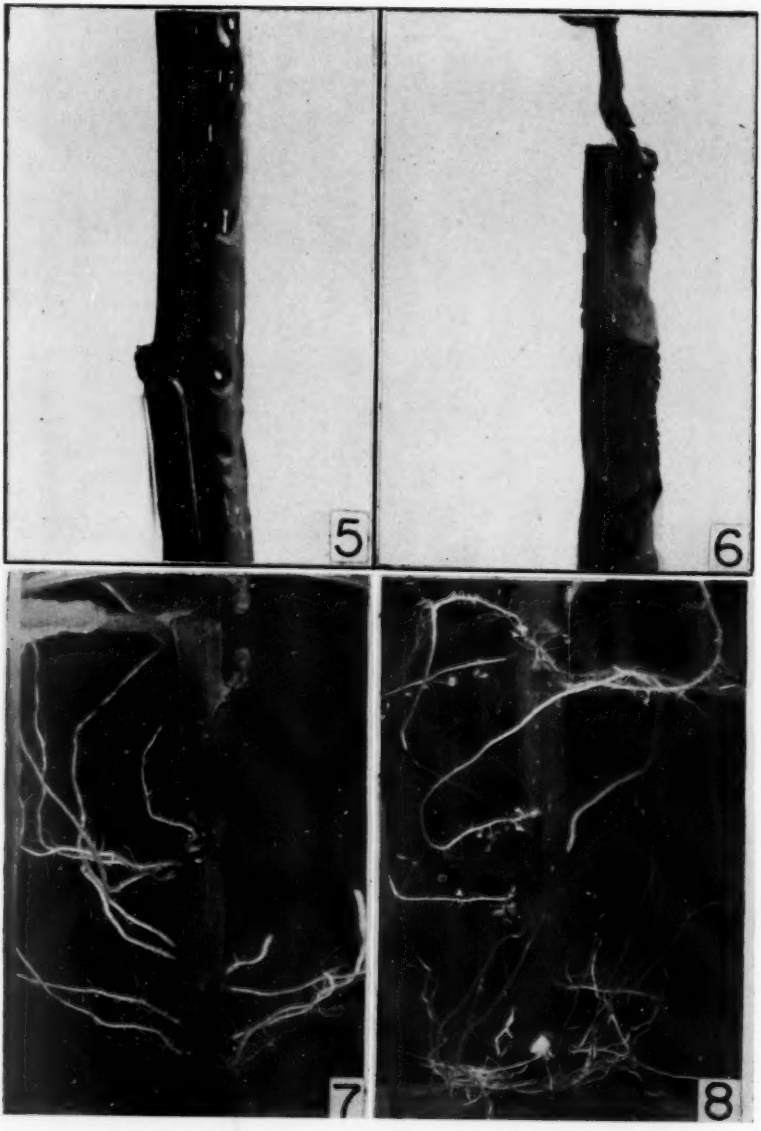
PLATE II



from the basal portion of stems, this weight increase may have been related to the ascent of sap which occurs prior to the end of the period of "imposed" dormancy as was described by Richardson (6).

Rooting Behavior in Uniform-sized Cuttings from Major and Minor Shoots

On February 3, 1960, 16 major and 16 minor shoots were harvested and stored outdoors for 10 days in an effort to obtain a broad range in bark moisture values. After the bark samples were taken, the cuttings were prepared and planted in clay pots containing vermiculate that were placed in shallow trays provided with a continuous supply of water at laboratory temperature. The root examinations were made 45 days after planting.

TABLE I

The relation of bark moisture to the weight and rooting of uniform-sized cuttings of *P.* 'robusta'

	Cuttings from major shoots	Cuttings from minor shoots
No. of cuttings	16	16
Weight of cuttings (g)		
Average	19.2	18.0
Range	17.8–21.8	17.5–18.9
Rel. turg. of the bark (%)		
Average	73	64
Range	65–76	60–72
No. of morphological roots		
Average	7.2	11.3
Range	2–15	8–17
No. of wound roots from basal callus		
Average	9.3	1.7
Range	5–15	0–8

As in the previous experiment the average level of bark moisture and the average weight were higher in the cuttings from the major shoots than in cuttings from the minor shoots (Table I). Although the latter produced the largest numbers of morphological roots developing from root primordia, the formation of basal callus was incomplete in many of these cuttings, which resulted in a limited production of wound roots, and the development of basal cankers caused by *F. lateritium* and *C. chrysosperma* (Figs. 5, 7). Cut-

FIG. 5. Multiple infections of *F. lateritium* on a cutting of *P. trichocarpa*, which failed to develop a complete basal callus and wound roots.

FIG. 6. Superficial mycelium of *C. chrysosperma* on a vigorously growing cutting of *P. trichocarpa*. Canker development did not occur on this type of cutting.

FIG. 7. Rooting in a cutting of *P.* 'robusta' with a lower level of bark moisture at the time of planting (rel. turg. 67%). Note absence of basal callus, wound roots, and the basal canker caused by *F. lateritium*.

FIG. 8. Rooting in a cutting of *P.* 'robusta' with a high level of bark moisture at the time of planting (rel. turg. 82%). Note the development of basal callus and wound roots.

tings from the major shoots produced fewer morphological roots, but many wound roots in association with well-developed basal calluses (Figs. 6, 8). Basal cankers did not develop on these cuttings. Similar results in the production of morphological roots were obtained when cuttings from major and minor shoots were placed in a dark, moist atmosphere with a temperature of 20° C.

From the experimental material it was apparent that the development of morphological roots was related to the incidence of root primordia, and was independent of degree of vigor as indicated by the ability of a cutting to produce a basal callus and wound roots. More morphological roots may be

FIG. 9. Region of the stems sampled for the cuttings derived from the major and minor shoots of *P.* 'robusta'.

expected on the cuttings from minor shoots since these were taken from the older parts of stems (Fig. 9). Therefore, in studies to relate the number of morphological roots with cutting vigor it would seem essential that the experimental material be derived from older stems in which all root meristem cells had become organized into typical root primordia, as has been mentioned by Shapiro (7).

A relationship was found between the bark moisture level in cuttings and their ability to produce basal callus and wound roots (Table I). Therefore, this aspect of cutting vigor was related to a qualitative rather than a quantitative expression of rooting.

The Relation of Bark Moisture and Total Weight to the Rooting of Uniform-sized Cuttings from the Major Shoots of P. 'robusta'

The results of the previous experiment suggested that it would be of interest to relate the bark moisture level and rooting in a more adequate sample of

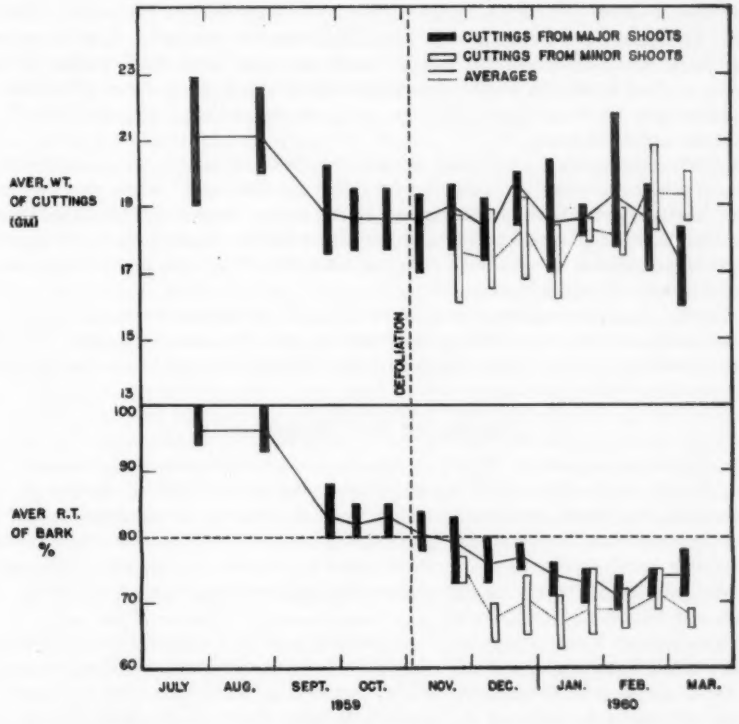


FIG. 10. Seasonal changes in bark moisture and total weight of uniform-sized cuttings of *P.* 'robusta'.

TABLE II

Relation of bark moisture to the total weight and rooting of uniform-sized cuttings from the major shoots of *P.* 'robusta'

Range in rel. turg. (%)	No. of cuttings	Av. rel. turg. (%)	Av. wt. of cuttings (g)	Average no. of roots per cutting		
				Morphological	Wound	All types
61–65	14	63.2	16.6	7.8	2.3	10.1
66–70	30	68.2	18.0	12.5	7.6	20.1
71–75	17	72.5	18.8	17.2	12.6	30.2
76–80	3	77.5	19.6	19.0	19.0	38.0
Simple correlation coefficients* for rel. turg. (%) on			0.545		0.636	0.678
for cutting wt. on					0.495	

*Statistically significant at the 1% level, exceeding r of 0.318 for 63 degrees of freedom.

cuttings taken from the same region of shoots, which would contain similar numbers of root primordia. Accordingly, 64 major shoots produced in 1959, were harvested on February 5, 1960, and stored outdoors until required. The bark moisture and total weight measurements, and the planting of the uniform-sized cuttings were undertaken over the period from February 5 to February 10. Root examinations were made on April 19, or from 69 to 74 days after planting.

Highly significant correlations were obtained between the total, and wound root production, and the relative turgidity of the bark when the cuttings were harvested from the same region of the major shoots (Table II). Incomplete production of basal callus and few wound roots formed on cuttings with relative turgidities of the bark ranging from 61–70%, and many were killed as the result of basal cankers.

Highly significant correlations were obtained between the total weight of cuttings of uniform size (25 cm long with a mid-diameter of 1 cm), and the bark moisture content, and the production of wound roots from basal callus (Table II).

Discussion and Summary

In previous studies (1, 2, 3) it has been demonstrated that the relative turgidity level in 1-year-old dormant bark was influenced by factors of the soil and (or) climate, and by cultural practices. Conditions adversely affecting tree vigor resulted in the lowering of the bark moisture levels and the development of a number of canker diseases caused by facultative parasites. Fungous attack was found to occur only when the relative turgidity of the dormant bark was below the 80% level.

Experiments with cuttings of *P. trichocarpa* and *P.* 'robusta' have shown a relationship between the relative turgidity level of the bark, and the formation of basal callus and wound roots. The production of basal callus and wound roots decreased in cuttings with relative turgidities of the bark below the 80% level, and this condition was accompanied by attack and mortality from stem canker diseases. Therefore, the bark moisture level could be used

to indicate the degree of vigor of a cutting based on rooting performance, in addition to its vulnerability to canker diseases caused by facultative parasites. The results provide direct evidence in support of the general concept that this type of disease occurs, for the most part, in trees of low vigor.

In a recent publication of the International Poplar Commission (5) it was mentioned that the percentage survival of cutting stock may be increased by a water-soaking treatment prior to planting. In the present study it has been demonstrated that the ability of cuttings to survive was closely related to the relative turgidity levels of their bark at the time of harvest. The provision of a continuous supply of water to cuttings over the period from harvesting to planting did not result in appreciable increases in bark moisture. However, it was possible to increase bark moisture to the original harvesting levels by supplying water to artificially desiccated cuttings. It is important to note that the cuttings with the lower levels of bark moisture at the time of harvest did not appreciably increase their bark moisture even when provided with a continuous water supply. Following planting, they demonstrated a low percentage of survival. The relative turgidity level, therefore, appeared to serve as an expression of the ability of the living cells in the bark to absorb and retain water, and may be expected to vary in cuttings in accordance with differences in the growth characteristics of the parent shoots.

In *P.* 'robusta' the largest numbers of morphological roots were produced on cuttings from the basal region of shoots, which agrees with the results of Bloomberg's experiments on *P. trichocarpa* (4). However, in the investigation of cuttings of uniform size from the basal and terminal regions of shoots it was demonstrated that the number of morphological roots was neither related to the bark moisture level nor to the percentage survival. Indeed, many basal cuttings which had produced the largest numbers of morphological roots were low in bark moisture at the time of planting and failed to survive because of their inability to produce basal callus. It was evident that the production of morphological roots was related to the stage in maturity of the stem tissues, the largest numbers having developed on cuttings from older parts of shoots, in which more root meristem cells had become organized into root primordia.

When planted cuttings of *P. trichocarpa* were placed in a moist chamber, superficial mycelium of *F. lateritium* and *C. chrysosperma* frequently developed at nodes and lenticels. However, basal and stem cankers occurred only on cuttings which failed to produce a complete basal callus and wound roots. The experiment demonstrated that the incidence of these canker diseases was related to host condition rather than to the presence or absence of viable inoculum.

In *P.* 'robusta' highly significant correlations were found between the weight of uniform-sized cuttings, and the bark moisture level, and the degree of cutting vigor based on the production of wound roots from basal callus. The possibility of applying average total weight as an indicator of the rooting potential of cutting stock could be of practical value. For example, a "hazard stick" technique might be developed in which an expression of the general vigor of dormant nursery stock or stored material could be obtained in the field by determining the average weight of cuttings of uniform size.

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THE SMUTS OF THE GENUS CINTRACTIA PARASITING KOBRESIA SPECIES[1]

ILKKA KUKKONEN[2]

Abstract

In this paper the modern concept of infection by species of *Cintractia* is briefly discussed. Three species are treated, two of which, *C. elynae* Sydow and *C. kobresiae* Mundkur, are redescribed, and the third is recognized as new, *C. lindebergiae* n. sp. The spore germination of *C. elynae* is described and illustrated. A short account of the host genus *Kobresia* is given with special reference to the species attacked by the above smuts.

As Savile (1952), Nannfeldt and Lindeberg (1957) and Lindeberg (1959) have shown, the ovariicolous *Cintractia* smuts parasiting many species of the genera *Carex* and *Kobresia*, and also *Trichophorum caespitosum* (L.) Hartm. (≡*Scirpus caespitosus* L.), form a natural and easily distinguishable group. Infection probably takes place during the short flowering period of the host and remains localized. The *Cintractia* spores most likely do not germinate during the first season. Thus they all must germinate on the ground the next spring, and we must suppose that the sporidia are the infecting agents. This conclusion agrees very well with the observations made by Savile (1952, p. 411) about the weather conditions in the areas where these smuts are common. Obviously, therefore, the life cycles of the host and the smut must fit closely together. Savile and Calder (1953) and Savile (1954) have pointed out another example of adaptation in echinosporous *Cintractia* species. The high warts provide the spores with a means of floating and, significantly, these species attack only aquatic *Carex* species. These observations give support to the conclusion that every *Cintractia* species infects only one host species or a group of closely related host species (see examples given by Brefeld 1895 and 1912, Sydow 1924, Liro 1938, Lehtola 1940, and Nannfeldt and Lindeberg 1957). In the following it will be shown that the smuts attacking the species in different sections of the genus *Kobresia* are morphologically distinguishable.

The genus *Kobresia* contains about 30 species according to Kükenthal (1909) or a few more according to Ivanova (1939). Only two of these, *K. myosuroides* (Vill.) Fiori & Paol., and *K. simpliciuscula* (Wahlenb.) Mack. are widely distributed. For the nomenclature of these species see Hylander (1955). *K. myosuroides* seems to be a holarctic species and is known also on the high mountains of the northern hemisphere. *K. simpliciuscula* is very similar in distribution but has, surprisingly, a gap in arctic Asia. Almost all of the other species are to be found only on the high mountains of central Asia. *K. schoenoides*, according to Kükenthal (1909) reaches the arctic coast

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in N.E. Asia. But, following the treatment of Ivanova (1939), we have to divide this species into several related taxa. The record of *Cintractia elynae* Syd. given by Liro (1938, p. 41) as on *K. schoenoides* accordingly has to be referred to *K. sibirica* Turcz. This latter species is known in arctic Siberia from the Urals to the Chukchi Peninsula. A specimen labelled *K. royleana* (Nees) Boeck. from the Hissar Mountains, Tajikistan, U.S.S.R., and infected with *Cintractia lindebergiae*, is probably actually *K. stenocarpa* (Kar. & Kir.) Steud. Ivanova calls the species from this area *K. stenocarpa* while the true *K. royleana* is a more southern species. *K. stenocarpa* and *K. capillifolia* and *K. laxa*, the other two species mentioned in this paper, all have a restricted distribution.

To get an idea of the relationships between the six *Kobresia* species to be discussed it may be worth noting the sections of the genus into which Kükenthal classifies them. Kükenthal divides the genus *Kobresia* into four sections: (I) *Elyna*, (II) *Hemicarex*, (III) *Eukobresia*, and (IV) *Pseudokobresia*. *K. capillifolia*, *K. myosuroides*, and *K. sibirica* belong to the first, and all the others, *K. laxa*, *K. simpliciuscula*, and *K. stenocarpa*, belong to his third section. *K. laxa* is, however, less closely related to the other species of this section as is evident from its transfer to the genus *Schoenoxiphium* by Ivanova.

Kobresia is generally agreed to be an ancient genus. It was suggested by Savile and Calder (1953) that the whole genus *Carex* was derived from some ancestral *Kobresia* species whereas Nelmes (1952) would derive a part of *Carex* from it. If our assumption about the close relations between the parasite and the host as given above holds, it should, at least, be possible to separate the smuts attacking the hosts in different sections of *Kobresia* and this was actually readily done. It was easily demonstrated that *K. myosuroides* and *K. simpliciuscula* are infected by different smuts, and it is significant that *K. sibirica* takes the smut of its close relative *K. myosuroides* whereas *K. stenocarpa* takes the one that infects its close relative *K. simpliciuscula*.

When studying the smuts infecting *Kobresia* species at the Mycological herbarium of the Plant Research Institute, Department of Agriculture, Ottawa, Canada (DAOM), I observed at first that in some specimens the spores had clear papillae on the rim of the flattened spores (see Fig. 3). Under the phase contrast microscope it was evident, however, that the papillae cover the spores all over, including the flat sides. Furthermore it was noticed that the form infecting *K. simpliciuscula* had papillate spores whereas the one infecting *K. myosuroides* had spores almost perfectly smooth. These characters were found to remain constant through the entire range of specimens examined. All spore measurements given in this work refer to material mounted in heated lactophenol.

Specimens from the following herbaria have been studied. Naturhistoriska Riksmuseet, Stockholm, Sweden (S), Botanical Museum of the University, Helsinki, Finland (H), Institut für Spezielle Botanik der Eidg. Technischen Hochschule, Zürich, Switzerland (ZT), Katedra Botaniky, University Karlovy, Praha, Czechoslovakia (PRC). The type of *Cintractia kobresiae* Mundkur has been borrowed from the Herbarium Cryptogamiae Indiae Orientalis, New Delhi, India (HCIO). Many specimens are now deposited also in the Botanical Museum, Department of Botany, University of Turku, Turku, Finland (TUR).

My sincere thanks are due to Dr. S. Ahlner (S), Dr. H. Roivainen (H), Dr. E. Müller (ZT), Dr. Zd. Urban (PRC), and Dr. R. Vasudeva (HCIO) for lending specimens. Especially I am indebted to Dr. D. B. O. Savile for his continuous interest in my work and for all of his help given during the progress of this study. Some important collections were made during the arctic field trip of the IX International Botanical Congress held in Montreal 1959, including the type of *Cintractia lindebergiae*. I am indebted to the Plant Research Institute for the opportunity to join the most interesting and successful arctic trip. I would like to include here also my acknowledgement to National Research Council for making my stay at Ottawa possible. My sincere thanks are due to Dr. J. W. Groves for his careful reading of this manuscript. Dr. B. Boivin has assisted in preparing the latin diagnosis.

Cintractia elynae Sydow

Ann. Myc. 22:289. 1924.

Syn.: *C. hyperborea* Ciferri, Ann. Myc. 29:63–64. 1931.
C. chinensis Yen 1935 (description seen in Yen 1937, see also Ling 1953, p. 335).

Type: Sydow in his paper does not cite any specimens. The only note he gives is that the smut attacks *K. myosuroides* in Switzerland. Fortunately I have seen a specimen from his herbarium which he almost certainly had seen at that time. That specimen, collected by Magnus in "Schweiz, Horald bei Zermatt", 29 Aug. 1886 (S) is designated as lectotype.

The description of *Cintractia elynae* given by Sydow is quite insufficient. Somewhat better is that given by Liro (1938, p. 40), who says that the spores are smooth. But he did not observe that the spores of the smut on *K. simpliciuscula* were papillate. Furthermore, the fungus on "*Elynanthus antarctica* Benth. & Hook." reported as *C. elynae* by Liro (1938, p. 41) has most probably nothing to do with smuts on *Kobresia* species. According to Harriot (1900) the collection cited was made by Dr. Hyades in "Baie Orange (Terra de Feu)". No species of *Kobresia* is so far known from Tierra del Fuego.

Ciferri (1938, p. 258) in dividing the *Kobresia* smuts into two species, has been misled by the complexity of the nomenclature of the hosts. The same is true of Zundel (1953). Fischer (1953) mistakenly cited the *Kobresia* smuts both under *Cintractia caricis* and *C. carpophila*. Savile (1952) has included them in his var. *elynae* (Syd.) Savile of *C. carpophila* (Schum.) Liro. The type host of *C. carpophila* is according to Liro (1938) *Carex caespitosa*. The smut on this host should probably be included in *C. heterospora* Lindeberg (see Nannfeldt & Lindeberg 1957, p. 500). It seems best to restore the species of Sydow. The disposition of the smuts on the other hosts mentioned by Savile under var. *elynae* falls outside the scope of this study.

Savulescu (1957) recorded *Cintractia elynae* for Roumania and gave a good but incomplete description.

The following description of *C. elynae* Syd. is drawn up using the method adopted by Savile (1952) for describing the smuts of *Cintractia*:

Spores more or less disc-shaped, (14)16–22(25) μ in diameter in face view, and (9)10–20(22) μ in side view, brown to blackish brown. In face view

wall seldom dotted, the dots ca. 0.1–0.3 μ in diameter and spaced ca. 0.4–1.5 μ. In side view wall occasionally slightly roughened on flat side, the rim always perfectly smooth. The spores are usually covered by a gelatinous sheath, best seen in side view, ca. 0.3–0.7 μ thick on the rim and ca. 1.0–1.5(2.0) μ thick on flat side. The dots seen occasionally on the flat side in face view as well as the roughness seen in side view are probably due to the differences in density of this gelatinous coat. Wall ca. 1.0–2.5(3.0) μ thick, thinnest on flattened face (ca. 1.0–2.0 μ) and thickest around the rim (1.5–2.5(3.0) μ), sometimes unevenly thickened and giving a slightly angular appearance.

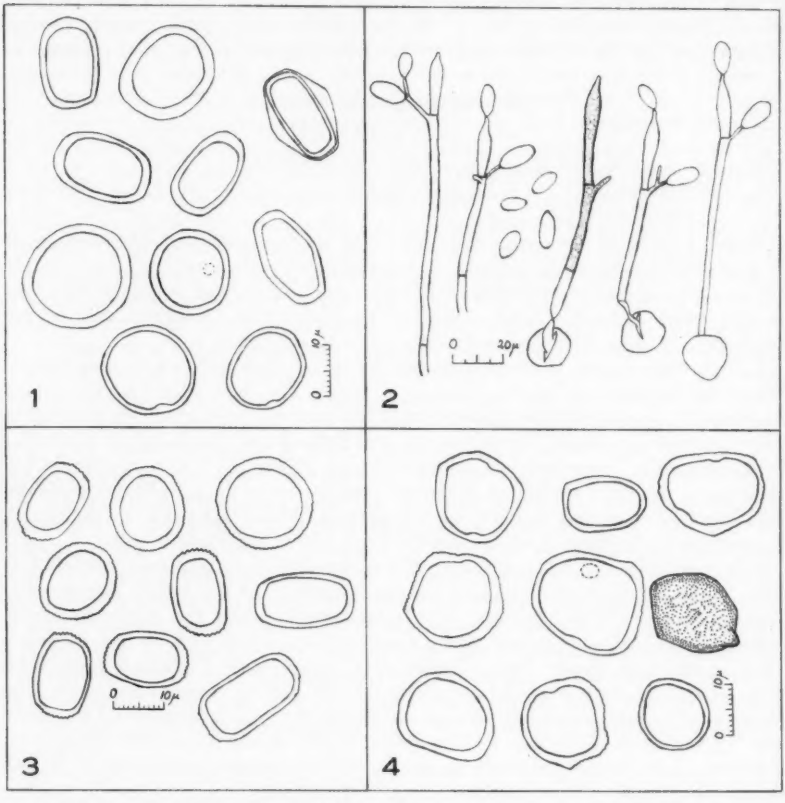

FIG. 1. Spores of *Cintractia elynae* Syd. In upper right corner a spore with gelatinous caps.

FIG. 2. Germinated spores, promycelia, and sporidia of *Cintractia elynae* Syd. In the middle a three-celled promycelium with two living cells.

FIG. 3. Papillate spores of *Cintractia lindebergiae*. Papillae best seen on the rim of the flattened spores.

FIG. 4. Spores of *Cintractia kobresiae* Mundkur. In the middle right, a spore is seen in face view showing the rows of papillae.

Often with 1–2(3) weak internal swellings (e.g. 0.5 × 1.5–2.0 μ) best seen as dark areas in face view (Fig. 1). In germination, spores form a promycelium, ca. 70–100 μ long, the lower cell ca. 3.5–5.0 μ thick. The promycelium consists usually of only two living cells, upper cell ca. 25–40 μ long and 4.0–5.0 μ thick. Sterigma ca. 4.0–8.0 μ long and ca. 2.0–2.3 μ thick. Sporidia ca. 6–17(18) μ long and 4–9 μ thick, with a mean 11.5 × 6.1 μ, ellipsoidal or varying from ovoid to obovoid. Each cell forms only one sporidium at a time, but later on a second sporidium will be formed from a secondary branch of the primary sterigma (see Fig. 2).

Specimens Seen

On *Kobresia myosuroides* (Vill.) Fiori & Paol. (=*K. bellardii*, *Elyna bellardii*)

Lectotype

Switzerland, Horald bei Zermatt, 29.8.1886, P. Magnus, ex Herb. Sydow (S).

In exsiccatae

Sweden, Lule Lappmark, Virijäurslätten, Vestergren; in Vestergren: Microm. rar. sel. No. 323 (S).—*Sweden*, Lule Lappmark, Jokkmokk parish, Nannfeldt; in Lundell and Nannfeldt: F. exs. suec. No. 643 (S).—*Sweden*, Torne Lappmark, Kiruna, Santesson; ibid. No. 2452 (S).—*Switzerland*, Zermatt, Körnicke; in Zillig: Ustilagineen Europas No. 125 (S).

Other Specimens

Canada:

Labrador (Newfoundland): 52 mi WSW. of Hebron. Gillett 8983 (DAOM).

Quebec: Sugluk, 62° 15′ N.: 75° 38′ W., Malte (H).

District of Franklin: Frobisher Bay, Baffin I., Calder 2163 (DAOM).—*ditto*, Calder 2194 (DAOM).—ditto, Savile, Calder & Kukkonen (DAOM, TUR).—Harbour Fjord, Ellesmere I., Simmons (H).—Eureka —, Ellesmere I., Bruggeman 880 (DAOM).

Yukon: Keno Hill, Calder 4270, Gillett *et al.* (DAOM).

British Columbia: Murray Range, Pine Pass, Calder 14069 Savile & Ferguson (DAOM).—Ashnola Range, 49° 04′ N.: 120° 12′ W., Calder 19596, Parmelee & Taylor (DAOM).—Goat Mtn., 49° 58′ N.: 114° 42′ W., Taylor 3015 & Ferguson (DAOM).

U.S.A.:

Colorado: Leadville, Kelso 6905*a* (DAOM).

Greenland:

Groenlandia bor.—orient., Termometerfjeld, ca. 76° 45′ N., Lundager (DAOM).

Iceland:

N. Island, Hals, Kjellberg (S).—Islandia Occ., Borgarfjardarsyla, Biskupshekka, Hadac (PRC).—ditto Fverfell, Hadac (PRC).—ditto,

famra Fverfell, Urban (PRC).—ditto Raydarvatn, Urban (PRC).—ditto, Raydarlackur, Urban (PRC).—ditto, "Vodopád Botusa", Sonchova (PRC).

NORWAY:
Dovre, Kongsvold, Ahlberg (S).—ditto, Ahlberg (H).

SWEDEN:
Härjedalen, Axhögen, Hulting (S).—Jämtland, Åre, illegible (S).—LL (=Lule Lappmark), Lindblom & Ångström (ex Herb. normali, 1835–1864) (H).—LL, Sarjekfjällen, Vestergren (S).—LL, Virijaure, Vestergren (DAOM).—LL, vid Norra Sarjekglacieren, Vestergren (S).—LL, Jokkmokk, Björkman (S).—TL (=Torne Lappmark), Kebnekaiseområdet, Du Rietz (S).—TL, Karesuando, S-Peldsa, Roivainen (S).

GERMANY?:
Specimen seen in Herb. Dearness No. 3152. On the label a note by Dearness: "Germany".

FRANCE:
The only note given on the label "Lantoret", perhaps = Col du Lautaret, French Alps, Grenier (S).

SWITZERLAND:
Graubünden: Hubelboden über Cresta Alpe, 2300–2400 m, Magnus (S).—Graubünden: Hubelboden über der Cresta Alpe, Magnus (DAOM, S).—Wallis: Auf dem Riffelberg bei Zermatt, Wolf, ex Kneucker: Cyperaceae & Juncaceae exsiccatae No. 142 (PRC).—Wallis: Saas-Fee am Weg zum Kessjenjoch, Fischer (S).

AUSTRIA:
Tirol, Toblach, Sarlkofel, Bornmüller (S).—Tirol, Obergurgl, Vockeroth (DAOM).—Tirol, Saiser Alpe, Pospichal (PRC).

SIBERIA:
Yakutsk, Buckur, Nilsson 720 (S).—Jacutia arctica, ad Lenam inferiorem, insula Tass-Ary, 71° 45′ N.: 127° E., Petrovsky (DAOM).—Kamtschatka orientalis: Simina volcano, Novograblenof (S).

On *Kobresia sibirica* Turcz.
SIBERIA:
Yakutsk, Buchur, Nilsson (H). (See also Liro 1938, p. 41, as *K. schoenoides*.)

On *Kobresia capillifolia* (Decne.) C. B. Clarke
INDIA:
Kashmir, Burzil Chowki, 28 July, 1940, R. R. Stewart (HCIO, Fungi No. 7715). After finishing my studies on the smuts of *K. myosuroides* and *K. simpliciuscula*, I received this specimen for study. It puzzled me at first, because it seemed to fall between *C. elynae* and *C. lindebergiae*. It differs, however, from both of them. Most probably it should

be treated as a separate species, but the scant material, one head with two sori, does not allow that. Therefore it is temporarily left here.

Description of the spores.—Spores 15.5–21.1 × 10.5–19.0 μ, rounded, flattened, dark brown. Wall in face view dotted, dots ca. 0.3—0.5 μ in diameter, widely spaced. In side view, wall papillate both on the rim and on the face, papillae sometimes seen even better on the face, ca. 0.1–0.3 μ high. Wall ca. 0.7–2.0 μ thick, thickest around the rim, often unevenly thickened and giving the spores a slightly angular appearance. Often with gelatinous caps on the flattened sides. In a few spores one weak internal swelling was seen. (See also Mundkur 1944.)

Cintractia lindebergiae sp. n.

TYPE: On *Kobresia simpliciuscula*. On sea beach near airport, Frobisher Bay, Baffin I., District of Franklin, Canada. 11 Aug., 1959. D. B. O. Savile, J. A. Calder, & I. Kukkonen. TUR, isotype in DAOM.

Sporae ± discoidae, (14)15–20(22.5) μ diam., (9)10–19(20) μ crass., a brunneis atro-brunneae, papillis hyalinis vestitae. Paries ca. 0.7–2.5 μ crass., tenuior (ca. 0.7–2.0 μ) in facies, crassior (ca. 1.5–2.5 μ) in latere. In speciminis aliquot sporae munitae sunt 1(2) tumoribus internis, nonnunquam involutae vagina gelatinosa crassiore in facie.

Spores more or less disc-shaped (14)15–20(22.5) μ in diameter in face view, and (9)10–19(20) μ in side view, brown to blackish brown, covered with hyaline papillae. In face view papillae seen with difficulty on flattened side but more easily visible on the rim. In side view papillae more readily seen on the rim than on the flat side. Papillae ca. 0.3–0.7 μ in diameter, and spaced ca. 0.4–1.5(2.0) μ, in side view ca. 0.1–0.3(0.5) μ high. Wall ca. 0.7–2.5 μ thick, thinnest on flattened face (ca. 0.7–2.0 μ) and thickest around the rim (1.5–2.5 μ). In a few specimens spores have occasionally one (or two) very weak internal swellings, often (especially when young) with gelatinous caps on flat faces (Fig. 3). My repeated attempts to germinate this smut were without success.

The species is named in honor of the late Mrs. Brita Lindeberg, who has contributed so much to the knowledge of Ustilaginales in Northern Europe.

Specimens Examined

On *Kobresia simpliciuscula* (Wahlenb.) Mack. *(= K. bipartita, K. caricina)* Canada:

Quebec: E. shore of James Bay near Cape Jones, Johansen 72 (DAOM).—Cape Jones, 54° 37′ N.: 79° 41′–45′ W., Dutilly, Lepage & Duman 32628 (DAOM).—Great Whale River, Savile, Calder & Kukkonen (TUR, DAOM).—Hudson Bay post, Fort Chimo, Calder 2445 (DAOM).—ditto, Dutilly, Lepage & Duman 28028*a* (DAOM).

District of Franklin: Frobisher Bay, Baffin I., Savile, Calder & Kukkonen (type TUR, isotype DAOM).—ditto, Calder 2202 (DAOM).—Lake Harbour, Baffin I., 62° 49′ N.: 69° 45′ W., Malte (DAOM).—ditto, 62° 49′ N.: 69° 55′ W., Malte (DAOM).

District of Keewatin: Coral Harbour, Southampton I., Cody 1795 (DAOM). —ditto, Cody 1821 (DAOM).—ditto, Savile, Calder & Kukkonen (TUR, DAOM).—Chesterfield Inlet, W. of River, 63° 25′ N.: 90° 40′ W., Duman 1852 (DAOM).—8 mi SW. of settlement, Chesterfield Inlet, Savile 1444 & Watts (DAOM).—Yathkyed Lake on the Kazan R., 62° 30′–63° N.: 97°–98° 30′ W., A. E. Porsild (DAOM).

Manitoba: Fort Churchill, Gillett 2438 (DAOM).

U.S.A.:

Alaska: Nabesna Road, mile 91, Dutilly, Lepage & O'Neill 21479 (DAOM). —Anvil Hill, Nome, Lepage 23902 (DAOM).—Near Nugget Creek, Brooks Range, Jordal 2315 (DAOM).—White Mts., Headwaters of Sourdough Creek, Gjaerevoll 1013 (DAOM).

GREENLAND:

Groenlandia Occident., S. Disko, Godhavn, 69° 15′ N., A. E. Porsild (DAOM).—Groenl. Occid., Godthaab Fjord, Itivnera, 64° 22′ N., M. P. Porsild (DAOM).

NORWAY:

Dovre, Kongsvold, "Ands." (H).—Dovre, Ahlberg (H).—Dovre, Alta, Moe (DAOM).—Kongsvold, Juel (S).—Kongsvold, Blytt (H).—Kongsvold (Dovre Fjeld), 62° N., am Wege nach Drivstuen, 900 m, Baenitz (PRC).—Dovre, Kongsvold, Neuman (H).

SWEDEN:

Härjedalen, Stora Mittåkläppen, Haglund (S).—Jmt. (= Jämtland), Åre, Jahnke (H).—Jmt., Åre, illegible (S).—Jmt., Frostviken, Raudekfjället, Norrman (S).

SWITZERLAND:

Erlashaug, Bernina, Graubünden, Braun (Herb. Volkart) (ZT).

AUSTRIA:

Tirol, Brenner, Domin (PRC).

On *Kobresia stenocarpa* (Kar. & Kir.) Steud. (see Ivanova 1939), (*Matrix nova*)

U.S.S.R:

Tajikistan: "Prov. Samarkand, Alpes Hissar, Nomobad, in valle Kasandemir, 3100 m," ex J. Bornmüller: Plantae Turkestanicae, legit Fedtschenko, No. 710 (S).

Cintractia kobresiae Mundkur

Mycologia, 36:291. 1944

TYPE: On *Kobresia laxa* Nees (= *K. laxa* Boeck.) at Satpura nulla, above Skardu, Kashmir, Aug. 3, 1940, R. R. Stewart 20357 (HCIO: Fungi, No. 7717).

As far as can be seen from the type collection, which is the only known specimen and consists of only one head without any mature perigynia, the host determination seems to be right.

This interesting smut is readily distinguished from the other two described above. Because Mundkur's description is not quite satisfactory, it is worth pointing out in this connection the most significant characters.

Spores 14–21 × 11–18(19) μ, rounded or ellipsoidal and often slightly angular, brown to dark brown. Wall covered with hyaline papillae always easily visible. In face view papillae ca. 0.2–0.5 μ in diameter, spaced ca. 0.3–1.0 μ, forming clear, curved rows and sometimes fusing together. In side view wall clearly verrucose with papillae ca. 0.1–0.2 μ high and 0.3–0.7 μ wide. Wall ca. 0.7–1.5 μ thick, often with 1–2 prominent internal swellings of the size, for example, 1.0 × 3.5 μ (Fig. 4).

The spore size given by Mundkur is "12.1–17.7 μ in diameter with a mean of 15.4 μ". The size seems, however, to be about the same as that of the other two species above. The angularity of the spores and the clear rows of papillae as well as the presence of prominent internal swellings characterize this species very well.

Summary

The main characters for distinguishing the three species described above can be best illustrated by means of a key.

1. Spores smooth or, at least, the rim always perfectly smooth..... *C. elynae*
2. Spores not smooth
   - *a.* Papillae not or, at most, hardly visible on the flat sides both in face and in side view..... *C. lindebergiae*
   - *b.* Papillae visible on the flat sides
     - *x.* Papillae visible on flat side in face view but no rows can be seen (see p. 161) the smut on..... *K. capillifolia*
     - *xx.* Papillae form clear, curved rows on the flat side in face view.. *C. kobresiae*

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VERTICILLIUM DISEASE OF STRAWBERRIES[1]

P. K. BASU

Abstract

An intensive study was made of the morphology, cultural characters, and infection capabilities of 18 isolates of *Verticillium*. These were in part isolated from different *Verticillium* hosts occurring in the Niagara Peninsula and the balance were obtained as named stock cultures from different herbaria. In terms of their pathogenicity to strawberries, resistant and susceptible tomatoes, and eggplants, the isolates comprised a number of races whose virulence ranged from lethal to very mildly pathogenic. Under the experimental conditions, current-year runner plants of strawberries proved to be virtually immune from infection, in contrast to normal overwintered plants. The symptoms on the strawberry are variable and diagnosis should be confirmed by isolation of the pathogen, for which a technique was evolved. Morphologically the isolates varied considerably and in culture the color ranged from white through gradually deepening gray to black. At the extremes the differences were conspicuous but were so intergrading in the group as a whole that the isolates are regarded as belonging to one variable species which, because of priority, must be called *Verticillium albo-atrum* R. and B.

Introduction

In the Niagara Fruit Belt the strawberry is a very useful crop for interplanting in young orchards and as an early season source of revenue. It has its full share of diseases incited by foliar pathogens, viruses, and root parasites. Mildew, leaf spots, and fruit rots are presently adequately controlled by protective fungicides and viruses reasonably so by plant certification. Root diseases, on the contrary, are not under control and remain the chief limiting factor in strawberry production.

Root degeneration in strawberries is an exceedingly complicated problem. Truscott (22) first studied it in the Niagara Peninsula and demonstrated that several fungi present among the normal soil flora were highly pathogenic to local strawberry varieties growing in otherwise sterile soil. None of these fungi, however, has ever been found inciting even local epidemics on strawberries. Hildebrand and West (6) showed that the root-rot potential of Niagara soils could be removed by sterilization and modified significantly and predictably by organic addenda which were shown to modify also the microbiological balance of the same soils. This suggested that, if strawberries were grown in as extended rotations as possible and if, for planting, only plants grown on more or less virgin soil outside the Niagara area were used, a satisfactory measure of control should be achieved. In general this has proved to be the case, although locally the results are frequently disappointing and the economic life of the plantations is too often limited to a single year of fruiting.

Further advances in controlling strawberry root diseases seem to depend on the evolution of economically feasible treatments of chemical soil disinfection

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and the further resolution and control of the disease components. The latter is rendered very difficult because the symptomatology is confused and non-specific and, by the time the symptoms are expressed, lesioned roots are invaded by so many saprophytes that the primary parasites are crowded out or unrecognizable. However, during the last decade, as several new varieties began to challenge the supremacy long held by the variety Premier, the rapid killing in midsummer of newly established spring-planted strawberries has been reported with increasing frequency. From such plants *Verticillium* has been isolated with sufficient frequency to suggest that here we are dealing with *Verticillium* wilt. The present investigations, accordingly, were undertaken in the hope of elucidating the significance of *Verticillium* in the larger problem of root degeneration of strawberries in the area under consideration.

Materials and Methods

Verticillium Cultures

Initially nine isolates of *Verticillium* were obtained by culturing directly from naturally infected plants in the Vineland, Ontario, area. During the course of investigation four more isolates of *Verticillium* and a species of *Fusarium* were added to the list of cultures. In addition to these, cultures of *Verticillium albo-atrum* R. and B. and *V. dahliae* Kleb. were secured from the Centraalbureau voor Schimmelculture, at Baarn, Holland (CBS); the American Type Culture Collection, at Washington D.C. (A.T.C.C.); and the Plant Research Institute, Research Branch, Department of Agriculture, Ottawa (P.R.I.). Table I presents a list of the isolates, the hosts, and sources from which they were isolated. Throughout the text each of the isolates has been referred to according to the designation assigned to it in this list.

TABLE I

Isolates under investigation

Designation of isolates	Date of collection	Isolation from	Sources	Specific name where indicated
A	Sept. 1956	Apricot	Vineland	—
B	Apr. 1959	Strawberry	C.B.S.	*Verticillium dahliae* Kleb.
C	Sept. 1956	Cherry	Vineland	—
D	Apr. 1959	Dahlia	A.T.C.C.	*V. albo-atrum* R. and B.
E	Sept. 1956	Eggplant	Vineland	—
F	June 1958	Strawberry	Vineland	*Fusarium* sp.
G	Apr. 1959	Not known	C.B.S.	*V. albo-atrum* R. and B.
H	Apr. 1959	Strawberry	C.B.S.	*V. albo-atrum* R. and B.
I	June 1958	Strawberry	Vineland	—
J	Sept. 1956	Shiro plum	Vineland	—
L	Sept. 1956	Lonicera	Vineland	—
M	Aug. 1959	Maple	Vineland	—
N	Apr. 1959	Tomato	A.T.C.C.	*V. albo-atrum* R. and B.
O	Apr. 1959	Lettuce	P.R.I.	*V. dahliae* Kleb.
P	Sept. 1956	Peach	Vineland	—
Q	Apr. 1958	Raspberry	Vineland	—
R	Aug. 1958	Rose	Vineland	—
S	Sept. 1956	Sumac	Vineland	—
T	Sept. 1956	Tomato	Vineland	—
U	Sept. 1956	Strawberry	Vineland	—
P_p	Sept. 1956	Pepper	Vineland	—

The original dates of isolation and serial numbers of the cultures obtained from C.B.S., A.T.C.C., and P.R.I., wherever these were indicated, were as follows:

N—*Verticillium albo-atrum* Reinke and Berthold, A.T.C.C. No. 10933; O.S. Cannon, Utah, Agr. Expt. Sta., Logan. 1950. From tomato wilt.

D—*V. dahliae* Kleb. A.T.C.C. No. 7611. F. A. Wiess, strain 837, U.S. D.A. 1939. From dahlia wilt.

O—*V. dahliae* Kleb. P.R.I. No. 42-I-245A, 1942. Lettuce seed.

H—*V. albo-atrum* R. and B. C.B.S. strain 20, of Van den Ende; From strawberry; 1958.

Plants Inoculated

Experimental host plants which were inoculated included: strawberry (var. Pocahontas); tomato (vars. John Baer and Geneva 11); eggplant (var. Black Beauty) and peach (var. Elberta). Current-year runner plants of strawberries were collected from Vineland Horticultural Experiment Station and from a field known to be wilt free near Niagara-On-The-Lake. One-year-old plants of a virus-free clone of Pocahontas were secured from W. F. Allen Co., Salisbury, Maryland, U.S.A. Tomato seeds of John Baer variety and eggplant seeds were obtained from Stokes Seed Co., St. Catharines, Ont. Geneva 11 variety of tomato and peach seedlings were available at the Vineland Horticultural Experiment Station.

Isolation Techniques

Thomas reported in 1932 that *Verticillium* could be readily isolated from the crown of strawberries (21). Keyworth and Bennett (12) also isolated *Verticillium* from crowns but mentioned some difficulties in that method, especially with respect to the surface sterilization of the crown.

Our isolations from the crown region were not consistent and rarely free from contaminations. Small pieces of roots and petioles were surface-sterilized with 0.1% $HgCl_2$, washed, and plated on media. Only very rarely was *Verticillium* isolated from roots, but it was obtained fairly consistently from petioles. Better growth and more consistent results, however, were obtained when pieces of washed petioles were surface-sterilized by dipping them in 1% sodium hypochlorite solution (24) for 10–15 minutes and then, without further washing, plated directly on a water-agar medium. This method was found very satisfactory also for the isolation of *Verticillium* from the hypocotyl region of tomato and eggplant seedlings and has been adopted throughout the present work.

Preparation of Inoculum for Infection Studies

Three kinds of inoculum were prepared. A spore suspension was made in the usual manner from a sporulating culture by adding sterile distilled water to the culture tube and gently shaking it. Mycelial fragments were present in such a suspension but no effort was made to separate these. A second type of inoculum was made by macerating cultures grown on V-8 agar plants in 8-oz medicine bottles. About 100 ml of distilled water was added and, after vigorous shaking, the contents were poured out and macerated in a Waring blender. A slurry liquid containing spores, mycelium, and microsclerotia was

obtained, which was diluted about 4 times before use. This inoculum will be referred to in the text as macerated fungus inoculum. A spore suspension or a macerated fungus inoculum which contained about 10,000 spores per ml, was considered a standard concentration for inoculation purpose. For introducing the fungus into soil an inoculum was made by culturing it on sand–cornmeal medium for 15–20 days. This inoculum will be referred to as sand–cornmeal–*Verticillium* mixture.

Methods of Inoculation

The root-dipping technique was found to be most effective in inducing wilt, as was also the experience of Wilhelm (24), Ende (5), Robinson, and his co-workers (19). In this method, roots of the plants to be treated were first thoroughly washed and rubbed lightly with the fingers to make superficial wounds on their surface and then they were dipped in a spore suspension or in a macerated fungus inoculum. Dipping for 24 hours was satisfactory for strawberries, but this period was shortened to 1–2 hours with tomato and eggplant seedling inoculations. Treated plants were planted into sterilized soil. Frequently, the residual inoculum was diluted and poured on the soil around the base of the plants. For infesting soil with the fungus, either macerated fungus inoculum was poured into sterile soil and mixed with it at the rate of 300 ml per flat of soil or a predetermined amount of sand–cornmeal–*Verticillium* mixture was mixed with the soil.

To introduce a spore suspension directly into plant tissues, two methods were employed. One was simply by direct injection with a hypodermic needle and the other was by flooding the intercellular space of tissues with a spore suspension following a partial evacuation.

Another method was used to inoculate young strawberry runner plants through stolons by keeping the cut end of the latter in a spore suspension in the following way: Stolons, about 12–15 inches long with a young runner plant attached to each, were cut under water; the cut end was introduced into a spore suspension in a specimen jar which was placed in the center of a flat containing sterilized soil. The runner plants were pegged on the soil to avoid unnecessary movement.

Preparation and Sterilization of Soil

Sand, peat, and loam soil were separately autoclaved for 3 hours at 15 lb pressure and this was repeated a second time after an interval of 24–30 hours. These components were then mixed in equal proportions, moistened, and stored in large tin cans for at least 4 days before use to avoid the toxic effect that freshly sterilized soil had been found to exert on transplanted tomato seedlings. This soil mixture has been the one usually used throughout the experiments. Occasionally, the 'John Innes Compost for potting', the composition of which follows, was also used: medium loam 7 parts, peat 3 parts, and sand 3 parts. Each ingredient was autoclaved separately and then mixed. To each bushel of the above mixture 0.75 oz of limestone, 1.5 oz of ground hoof and horn, 1.5 oz of superphosphate, and 0.75 oz of potassium sulphate were added.

Recording Instruments and Other Equipment

Measurements of available soil moisture in the field and in pots were first

recorded by a Bouyoucos moisture-meter with plaster of paris blocks buried 4 inches in the field soil and at suitable depths in pots. Great difficulty was encountered, however, with plaster of paris blocks inasmuch as once the blocks became saturated with moisture the meter continued to register 100% available moisture for an extended period thereafter, even though the soil seemed to be very dry. This difficulty was partially circumvented by using an electrical conductivity method which recorded the electrical resistance between two points in the soil, by inserting in it two electrodes mounted at a fixed distance. The two electrodes were connected to a volt–ohm–milliammeter (V-O-M. meter) which registered the resistance in ohms. The V-O-M. meter was calibrated against the readings of Bouyoucos moisture meter in terms of available moisture in soil. Therefore, whenever resistance became higher than 6000 ohms, indicating less than 100% available moisture, water was added to lower the reading to less than 6000 ohms. In this way one could be virtually certain that a soil moisture deficiency was not responsible for whatever wilting did occur.

The atmospheric temperature and relative humidity were recorded by hygrothermographs in greenhouses.

For growing plants under controlled conditions, Wisconsin soil temperature tanks, temperature-regulated growth chambers, and a mist-propagation bench were available at the Department of Botany, University of Toronto.

Cultural Studies of the Isolates of *Verticillium*

Selection of Media

Isaac (8) obtained best growth of his isolates in Dox's, Czapeck's, potato-dextrose agar (P.D.A.) while Chowdhuri (4) and Robinson *et al.* (19) used P.D.A. In the present study, each isolate was grown first on the following agar media; V-8 juice (15), potato-dextrose, lima-bean, 2% malt, modified Leonian's, and Czapeck–Dox's. The best growth and sporulation of all the isolates were obtained on V-8 juice agar and modified Leonian's agar medium and, since it was so readily available and easily prepared, the V-8 juice agar medium was selected for culturing the isolates throughout the study. The pH of this medium after autoclaving was 6.9 and this is well within the optimum range of pH for growth of *Verticillium* as recorded by many workers (8, 19, 16, 5).

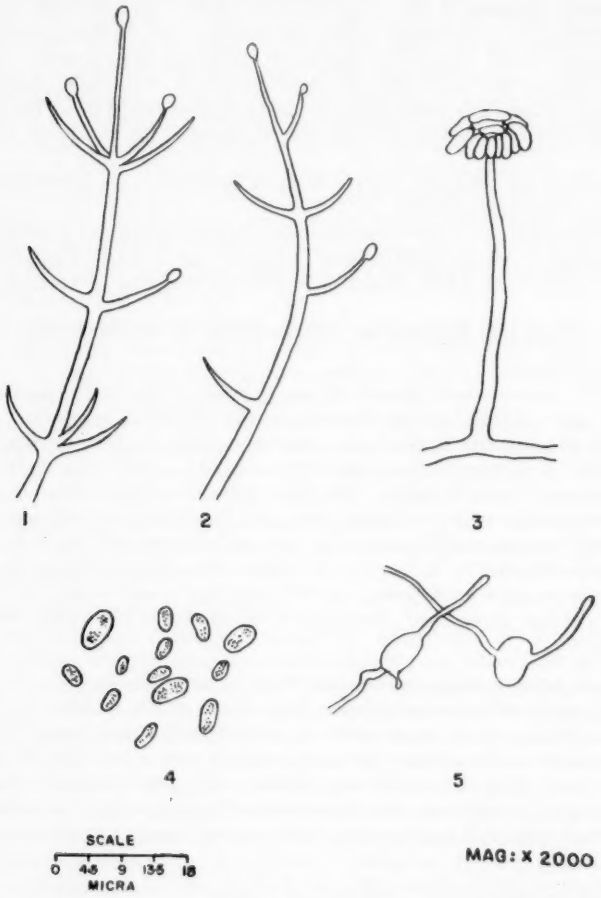
Comparison between Monosporous and Polysporous Cultures

Monosporous cultures were made from each of the isolates by the usual dilution technique and these were compared with the corresponding polysporous cultures. The amount of each transfer was standardized by using a 'biscuit cutter' type of transferring needle. No apparent differences between the two types of cultures were encountered. Accordingly, polysporous culture, derived initially from monosporous cultures, were used in routine culture operations.

Growth and Morphology

For comparison of growth and morphological characters each isolate was grown separately, as well as in association with two different ones on 9-cm

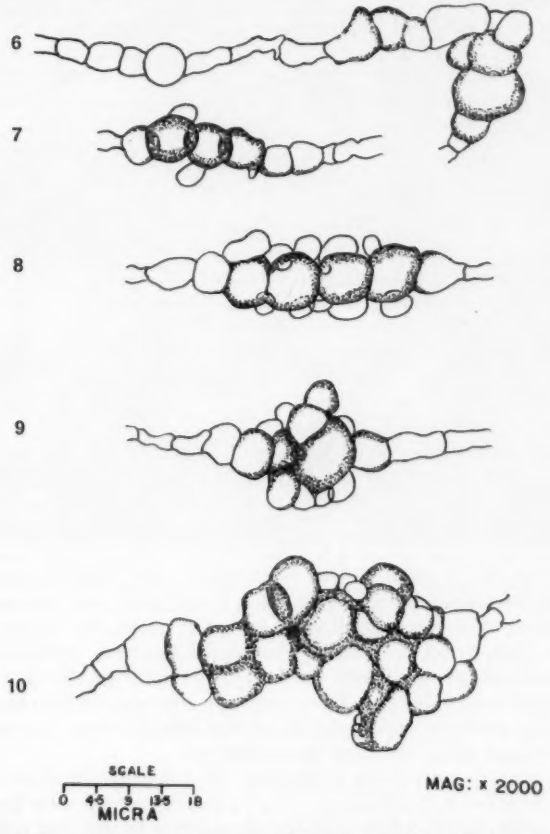
petri plates. Transfers were made by the 'biscuit cutter'. Equal transplants of three different isolates were placed 15 mm from one another in the form of a triangle on one plate to observe associative effects. Three similar transfers of each isolate similarly placed on petri plates served as checks. Two replicates of each plate were run. Each of the following isolates, A, B, C, D, E, H, I, J, L, M, N, O, P, Q, R, S, T, and U, came in association with another isolate at least 9 times in different ways. This was to observe the mutual reaction between two isolates placed side by side and also to note whether the presence of



FIGS. 1 and 2. Typical verticillate and irregular conidiophores of *Verticillium*. FIG. 3. Simple unbranched conidiophore with conidia at the tip. FIG. 4. Conidia with variable size. FIG. 5. Germination of conidia.

a third one made any difference. A parallel series of five replicated cultures of each isolate was carried in culture tubes. All the cultures were incubated at 22° C. Periodic observations were made for 6 weeks, and the average colony diameter of each isolate on 8th day is presented in Table II. The maximum average growth (22.7 mm) was attained by the isolate O, and the minimum (18.2 mm) by the isolate U, but the difference between them was not significantly different from the variation encountered among the replicates of individual isolates. Thus there was no apparent antagonism between the isolates on V-8 juice agar. This absence of antagonism and growth of some of the isolates are shown in Fig. 11.

Macroscopically, the isolates could be arranged in a gradually intergrading series ranging from a jet black color, with very little aerial mycelium, to com-



FIGS. 6–10. The gradual development of microsclerotia by thickening and darkening of cell walls in a single hypha and the subsequent budding of new cells.

pletely white forms with fluffy aerial mycelium. The intermediate forms were dark to light grey in color with variable amounts of white aerial mycelium. On microscopic examination, the relative darkness of the cultures proved to be due only to different amounts of microsclerotia and not to the presence of dark carbonized hyphae. Culture G was an exceptional form, having a mucoid type of growth, without microsclerotia and with scanty appressed mycelium. In general, the cultures which produced abundant microsclerotia usually had less aerial mycelium and vice versa. The completely white forms usually did not produce any microsclerotia, except as a result of special treatments which will be described later. Except for the presence or absence of microsclerotia, the isolates did not differ morphologically to any great extent. In the initial stages of the growth of a colony only white hyphae were produced, some of which were prostrate, growing along the surface of media, while others were aerial. Prostrate and aerial hyphae were alike in all the isolates. They were always hyaline, septate; usually 2.1 to 2.8 μ in diameter, though rarely 1.4 μ or 3.5 μ. No darkening of the hyphae occurred until the microsclerotia began to form. Sporulation occurred profusely within 4–5 days and the conidia were broadly to narrowly elliptical in shape (Fig. 4). The range of conidial measurements is given in the Table II. Except for isolates I, O, B, and P, the conidial size most frequently encountered was 2.8 $\times$ 5.7 μ. Isolates I and O usually formed narrowly elliptical conidia which measured 1.4 $\times$ 5.0 μ, while isolates B and P produced broadly elliptical conidia 1.8 $\times$ 3.6 μ in size. Conidia of isolate B were slightly smaller than those of P. Such small differences in the size of conidia among the different isolates cannot be regarded as very significant in view of the wide range of conidial size encountered in all of the isolates. Germination of conidia was observed in water in hanging drop cultures (Fig. 5). The mode of branching of conidiophores was variable. Although the verticillate arrangement (Fig. 1) was common in all isolates, 'alternate' and 'opposite' branchings (Fig. 2) were encountered, as well as simple unbranched conidiophores with a group of conidia at the tip (Fig. 3).

The development of microsclerotia was alike in all cases, although the time required for their appearance varied somewhat in different isolates, as is indicated in Table II. The shortest time recorded for their production was 4 days and the longest period was 45 days. In the initial stage of development of microsclerotia, some portion of a hypha became broader and closely septate, thus forming a row of more or less rectangular cells (Figs. 6 and 7), which gradually became darker and thick-walled. From these cells new cells budded off in all planes; these were hyaline at first, but gradually became dark and thick-walled (Figs. 8 and 9). Finally, the microsclerotium consisted of a mass of thick-walled cells among which the outer dark walls of adjacent cells could no longer be resolved (Fig. 10). These aggregations appeared to remain intact indefinitely. No germination of microsclerotial cells has been encountered, nor have other hyphae been traced to them directly.

It may be mentioned in this connection that 3-year-old dried cultures of *Verticillium* (isolates A, C, E, J, L, S, T, P_D and U) which were kept at room temperature grew afresh when transferred to fresh media and retained their pathogenic characters when tested on tomato plants. Similar viability has also been recorded by Isaac (7). However, it was difficult to decide from which

PLATE I

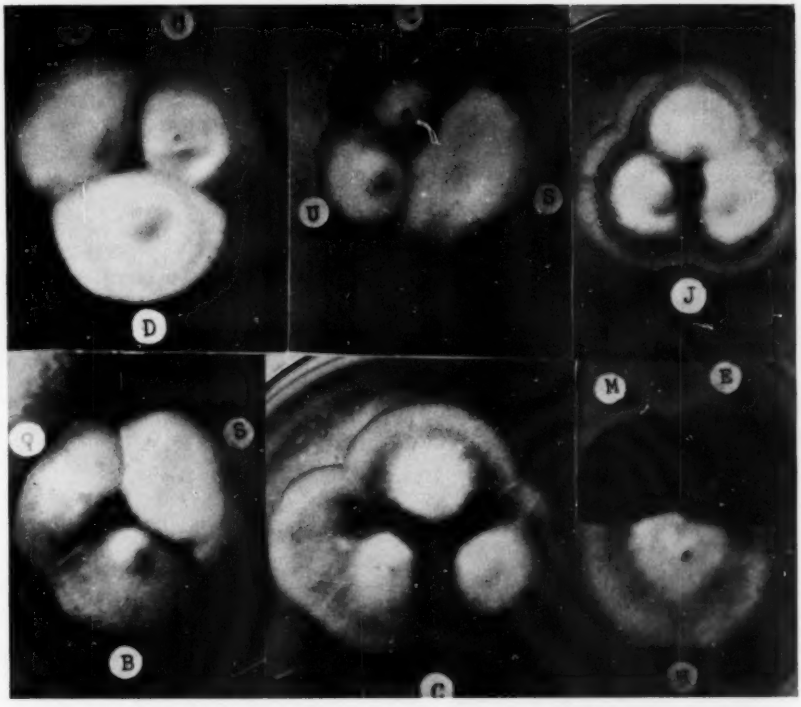


FIG. 11. *Verticillium* isolates showing (*a*) varying degrees of color from pure white (D) to black (E) and (*b*) the absence of antagonism when three different isolates are growing on the same plate. Two plates contain three transfers of isolates C and J. ×0.8 approx.

part of the old culture new growth started. Germination of microsclerotial cells has not been encountered in the present study nor has it been reported in the literature. The viability of conidia on dried media for 3 years at room temperature seems improbable and, therefore, old hyphae associated with microsclerotia appear to grow afresh as suggested by Ende (5).

Some associative effects were noted in relation to the production of microsclerotia. Some isolates (e.g. S, O, I, and A) which normally produced only a few microsclerotia after a long period and some other like T, which did not usually produce them at all, started producing microsclerotia sooner and in larger amounts when grown in association with a typical microsclerotia-producing isolate, like B, C, or E. Isolates D and U never produced microsclerotia on V-8 juice agar medium, even in association with microsclerotia-producing isolates. However, isolate U did produce microsclerotia in moderate amounts as long as it was grown on autoclaved potato plugs, whereas isolate D did not do so under similar condition. Apparently among the isolates used in this study only D lacked completely the ability to produce microsclerotia, once this was lost. When isolate D was first received it did produce a small amount of microsclerotia in culture but, after a few transfers, this ability was lost and never regained. The restoration of microsclerotia formation by potato plug culture has been reported also by other workers (20, 19). Formation of microsclerotia by the non-microsclerotial isolate G has been encountered after a passage through strawberry and this character was retained through several subculturings in V-8 juice medium. This is the only instance in this study where a host seemed to influence the capacity of a certain culture to produce microsclerotia.

A summary of the morphological characteristics of the isolates of *Verticillium* is given in Table II.

TABLE II

A summary of morphological and cultural characteristics of the isolates of *Verticillium*

Isolate	Range of conidial size in microns	Amount and time of microsclerotia formation	Average growth in mm on 8th day
A	(2.8–8.5) × (1.4–5.7)	Scanty; 12 days	20.8
B	(2.2–7.2) × (1.3–2.7)	Abundant; 4–5 days	21.7
C	(2.1–8.5) × (1.4–5.7)	Abundant; 4–5 days	22.0
D	(2.8–8.5) × (2.1–4.2)	Nil —	21.3
E	(2.8–8.5) × (1.4–3.5)	Abundant; 4–5 days	21.1
G*	(2.8–8.5) × (1.4–4.2)	Nil —	—
H	(2.8–8.5) × (2.1–3.5)	Medium; 12 days	21.4
I	(2.8–8.5) × (1.4–2.8)	Scanty; 12 days	21.5
J	(3.5–8.5) × (1.4–5.0)	Abundant; 5–7 days	20.7
L	(3.5–8.5) × (1.4–3.5)	Abundant; 5–7 days	20.7
M	(3.5–8.5) × (1.4–3.5)	Abundant; 4–5 days	20.5
N	(3.5–8.5) × (1.5–3.5)	Scanty; 20 days	20.3
O	(2.8–8.5) × (1.4–2.8)	Scanty; 12 days	22.7
P	(2.8–5.7) × (1.4–2.8)	Abundant; 5–7 days	21.0
Q	(3.5–8.5) × (1.4–3.5)	Abundant; 5–7 days	20.8
R	(3.5–8.5) × (1.4–3.5)	Abundant; 5–7 days	20.0
S	(3.5–8.5) × (1.4–3.5)	Scanty; 12 days	21.7
T	(2.8–8.5) × (1.4–2.8)	Scanty; 30–45 days	20.0
U*	(2.8–8.5) × (1.4–2.8)	Nil —	18.2

*Isolate G produced microsclerotia after one passage through strawberry plants; isolate U produced microsclerotia only when grown on potato plugs.

These cultural studies with different isolates of *Verticillium* may be summarized as follows: The differences in the color of colonies, which ranged from jet black to pure white, were found to be due to different proportions of microsclerotia and white aerial hyphae. Microscopically the isolates were almost identical, with respect to the hyphal and conidiophore characters, the range of conidial size, and the mode of development of microsclerotia. The isolates did not show any antagonism when grown in association with one another. In some instances the ability to form microsclerotia seemed to have been acquired due to an associative effect and this ability could also be restored by using suitable media or by passage through a suitable host.

Confusion exists regarding the nomenclature of pathogenic isolates of *Verticillium*. The first description of a pathogenic form of *Verticillium was* given by Reinke and Berthold in 1879. They named it *Verticillium albo-atrum*. Thirty-four years later Klebhan isolated another pathogenic form of *Verticillium*, which he considered distinct from *Verticillium albo-atrum* R. and B. and which he named *Verticillium dahliae*.

Following the establishment of this new species, *Verticillium dahliae* Kleb., taxonomic controversies regarding its validity arose and are still continued. These relate to the correct interpretation of the drawings and description of *Verticillium albo-atrum* as defined by Reinke and Berthold. Extensive reviews have been made on the taxonomic position of *Verticillium albo-atrum* and *V. dahliae* by several workers. It is apparent from the review of literature (2, 3, 5, 9, 12, 13, 16, 17, 18, 19, 20, 25) that the weight of opinion is now about equally divided as to the validity of the species *Verticillium dahliae* Klebhan. From all the evidences at hand the present writer is disinclined to support the separation of the two species and considers all isolates under present investigation as belonging to one species, namely, *Verticillium albo-atrum* R. and B., because this name has priority. A more detailed discussion of this subject is contained in the thesis (1) on which this paper is based.

Infection Experiments with Strawberry Runner Plants

It seemed logical to begin the strawberry infection studies with young unrooted runner plants growing from healthy mother plants, especially since this made it easy to avoid the possibility of previous infection from soil. An isolate of *Verticillium* from strawberry (isolate U) was used for the initial inoculations, and subsequently other isolates were incorporated.

Experiment 1: Inoculation by Dipping Stolons in Spore Suspension

Fifty young unrooted runner plants, each with a 12-in. to 15-in. stolon, were collected by cutting the stolons under water to maintain the continuity of the vascular stream. A specimen jar containing a spore suspension of isolate U was placed in the middle of a 1 ft × 2 ft flat which was then filled with steam-sterilized soil. Twenty-five runner plants were pegged to the soil with U-shaped pins, while the cut end of the stolons remained submerged in the spore suspension. A similar setup was maintained as check with the other 25 plants whose stolons dipped into water. The two flats were placed in a shaded place in the greenhouse and the soil was kept moist by light watering.

The cut ends of the stolons in both inoculated and check plants repeatedly became plugged by a gummy secretion. Whenever this accumulated, a fresh cut was made to facilitate the entrance of the spore suspension or water into the vessels. Within a week, many of the plants began to dry up and the root initials of the runner plants did not grow further into the soil. The majority of the plants died before becoming established. This experiment was repeated several times, but the plugging of the cut end of the stolons and the difficulty in rooting of the runner plants were limiting.

Experiment 2: Inoculation by Injection into Stolon and Subsequent Infestation of the Soil

To avoid the difficulties encountered in the previous experiment, young runner plants were allowed to remain attached to mother plants for nutritional support and inoculations were made by injecting a spore suspension into the stolons at different places.

Ten healthy mother plants, each with six runner plants, were collected from the field and potted in the same soil in 8-in. pots. The runner plants were trained on and pegged to sterilized soil in 4-in. pots. Into three stolons from each mother plant about 1 ml of spore suspension was injected at different places and the wounds covered with sterile cotton, while into the other three stolons of the same mother plant, sterile water was injected to serve as checks. The setup was kept in the greenhouse.

The young runner plants produced roots and became established without difficulty but none of them showed any sign of wilting within 6 weeks. As the individual runner plants eventually became nutritionally independent of the mother plant, the stolons dried up in a natural way and the runner plants were then separated from the mother plants by cutting off the stolons.

About 8 weeks after the first inoculation, a second inoculation was made in all plants, except those which were kept as checks, in the following way: five to six holes were made in the soil around the base of each plant to a depth of 2–3 inches with a cork borer of $\frac{1}{4}$-in. diameter. In doing so, many of the roots must have been injured and this was believed to favor infection. Through these holes sufficient macerated fungus inoculum was added until the soil was saturated, after which they were filled with the surrounding soil.

No wilt symptoms appeared in plants thus inoculated within the next 8 weeks, although by that time the mother plants were less thrifty than the runner plants. This unthrifty condition was not necessarily related to *Verticillium* infection, because these plants had been growing in the field soil during the 4 months since these were collected.

It was clear from this experiment that the runner plants did not become infected either by absorbing a spore suspension through the stolons or through injecting a spore suspension into the stolons or by introducing the fungus into the soil in the region of injured roots.

Experiment 3: Inoculation by Root-dipping Method

In view of the negative results of infection in the previous studies, some experiments utilizing the root-dipping technique of inoculation were initiated. For this purpose, young rooted runners, growing in the field or in the greenhouse in a perlite bed under mist, were selected and 11 different isolates of

Verticillium (viz. A, C, E, J, L, I, O, P_p, S, T, and U) were used for inoculations. The inoculated plants were kept and observed under five different environmental conditions as follows:

(*a*) plants kept under ordinary greenhouse conditions,
(*b*) plants kept at different soil temperatures in four Wisconsin soil temperature tanks,
(*c*) plants kept in a constant environment at optimum temperature,
(*d*) plants kept in a constant environment at higher temperature,
(*e*) plants kept in a constant environment at lower temperature.

For maintaining constant temperature, humidity, and light, three controlled-growth chambers were used. The details of the experiments under (*a*), (*b*), (*c*), (*d*), and (*e*) are presented below:

(*a*) Two hundred and sixty runner plants were collected from the field and thoroughly washed. Twenty plants were inoculated with each of the nine isolates (viz. A, C, E, J, L, P_p, S, T, and U) by the root-dipping method. Eighty plants which were kept as checks were dipped in water. After inoculation, each plant was potted singly in a 4-in. pot containing sterilized soil mixture and the pots were kept in the greenhouse. This experiment was begun in August 1957.

Within 2–3 weeks, it was noted that many of the smaller plants, which had fewer than five main roots, did not transplant well, whereas those with better root systems became established without any setback. During the following 16 weeks, however, none of these plants showed any symptoms of wilt and in their general condition were essentially the same as the check plants. In all of the plants some of the outer leaves dried up and died, and their growth was very slow during the following winter months, a period when under normal conditions strawberry runner plants undergo dormancy. However, some flowering and fruiting continued during the whole winter.

With the advent of warmer and longer days in the spring of 1958, the plants started new growth and at this time a second inoculation was made by introducing macerated fungus inoculum in soil as described in experiment 2.

No infection resulted from this second inoculation nor from a third one made in similar way after repotting of the plants at the end of summer 1958. During the summer of 1959, these plants were removed from the pots and planted in the field in a plot fumigated with chloropicrin. From some of the plants the old soil ball was shaken off before planting, but these plants failed to become established and soon died. The plants which survived grew in a normal way without any symptoms of disease. Cultures were made from the petioles of these plants, but *Verticillium* was never reisolated. Therefore, the experiment was terminated after 2 years of observation during which three inoculations had been made at long intervals (viz. first in early fall of 1957; second in early spring of 1958, and third in late summer of 1958) without positive results.

The essentially identical behavior of the inoculated and check plants over this extended period is challenging, but its explanation is by no means apparent. So far as the first inoculation is concerned, the juvenile runner plants evidently possess some sort of resistance to infection. The transition to a susceptible state normally occurs during the fall and winter when the plants go into a dormant condition, from which they emerge in early spring to rapid vegetative

development and the early onset of flowering. When the plants were overwintered in the greenhouse, they grew slowly but did not become dormant and began flowering in December. They withstood the inoculations made before that time as well as those made subsequently, which seems to imply that they never became susceptible under these experimental conditions. This suggests the desirability of controlled studies on the influence of low temperature on the susceptibility to *Verticillium* of current season runner plants, if the onset of susceptibility is correlated, as it appears to be, with the dormant condition. This may prove a profitable field of investigation, which we have not as yet had an opportunity to explore.

(*b*) Following these negative results it was decided to use in the next experiment newly isolated cultures of *Verticillium* and a more dependable source of disease-free runner plants, achieved through propagating the young runner plants on a perlite bed under mist. Under continual mist the young runners never wilted and continued to develop new leaves and roots for many weeks. When a good root system having 5–8 main roots with numerous fibrous ones had developed, the plants were ready for inoculation.

To observe the influence of soil temperature on the infection of runner plants, four Wisconsin soil temperature tanks were used and these were maintained at the temperatures of 16° C, 20° C, 25° C, and 30° C respectively. Each tank had eight copper cans which were filled with sterilized soil mixture. Three plants could be planted in each can. Thus, depending upon the accommodation in each tank, nine plants inoculated with isolate I, which was newly isolated from naturally infected strawberry plants, were planted in three cans in each tank. Similarly, in each tank, nine plants inoculated with isolate O and six uninoculated check plants were planted.

A temperature of 30° C proved to be definitely injurious to the young runner plants, because within a week all the plants in that high temperature tank simply dried up. In all the other tanks, a few plants died within the same interval, but those which survived for 8 weeks or more grew slowly but showed no typical wilt symptoms. Here again the similarity in appearance between the checks and the inoculated plants made it difficult to interpret the results, because poor growth and stunting might, in some instances, be induced by low-grade or suppressed *Verticillium* infections, even though in general they reflected an otherwise unsuitable environment. However, it was significant that in none of these plants were new roots developed after they were transplanted. Due to the lack of positive symptoms, it was tentatively concluded that infection did not take place in the runner plants, under the different soil temperatures.

(*c*) In this experiment, 60 runner plants were selected from the plants grow ing under mist. Twenty plants were inoculated with each of the two isolates I and O, while 20 plants were kept as checks. The inoculated and check plants were potted in sterilized soil in 4-in. pots which were kept in three growth chambers in which temperature, relative humidity, and light were controlled. The temperature was adjusted to 22° C and the relative humidity to 72%. The illumination was of the order of 1150 foot-candles at the plant surface and the day length was 14 hours.

Once again nearly 30% of the plants did not establish properly and con-

sequently died within few days, but for the next 8–10 weeks more than 70% of the plants not merely remained alive, but grew considerably and produced new leaves and roots. During this period no wilting was observed and, as in the previous experiments, there was no significant difference between the inoculated and check plants.

(*d*) and (*e*) This experiment was designed to investigate the effect of higher and lower temperatures on inoculated plants. One of the growth chambers was maintained at 12° C and another at 28° C, whereas the conditions of light and relative humidity remained the same as in the previous experiments. In each of two flats of soil heavily infested with isolate E, 17 runner plants were planted following an initial inoculation with the same isolate by the root-dipping method. The infestation of the soil in flats was done by soaking the soil in each flat with 300 cc of macerated inoculum. Ten uninoculated plants potted in sterilized soil were kept as checks in each growth chamber.

No typical symptoms appeared within 8–10 weeks. Some of the outer leaves of the plants in flats had a prostrate appearance, with a general yellowing similar to that of normal senescent leaves. All the petioles and roots of inoculated plants and some of the check plants were examined for the presence of *Verticillium* by culturing them in the usual manner. From the 12° C chamber, no *Verticillium* was reisolated from 63 roots and 62 petioles of inoculated plants, nor from the check plants. From the 28° C chamber, *Verticillium* was reisolated from only 2 petioles out of 50 and 20 roots of inoculated plants and none was obtained from check plants.

From this experiment, once again it is the rarity of infection which is most conspicuous. It is suggestive, however, that the only place the fungus was obtained from plants, was at 28° C, which is well above optimum for both host and pathogen.

Experiment 4: Inoculation by Three Methods at the Same Time

Thirty unrooted runner plants with long stolons were collected from healthy mother plants. The runners were allowed to root under mist propagation bench. Fifteen plants were inoculated by root-dipping method with isolate E and then planted in soil heavily infested with the same isolates. A fresh cut was made at the end of each stolon, which was kept submerged in a spore suspension in the same manner as described for experiment 1. The experiment was set up in the latter part of July 1959 and was conducted under greenhouse conditions. A similar setup with uninoculated check plants was maintained.

With a very few exceptions, all the plants became established and remained healthy during the following 4 months without showing any definite wilt symptoms. Slight yellowing and a prostrate appearance of the outer leaves were observed but *Verticillium* was never reisolated from the petioles of any of the plants. This result again indicated the highly resistant nature of the young runner plants.

Experiment 5: Inoculation by Flooding Intercellular Space with Spore Suspension

Finally, one further attempt was made to infect the young runner plants by flooding the intercellular spaces of the tissues following a partial evacuation as described previously. In this experiment 15 runner plants were inoculated with isolate E. Ten plants were treated in the same way without the fungus.

Following inoculation, the plants were potted in sterilized soil and kept in the greenhouse.

All the plants became established and none showed wilting within 45 days. *Verticillium* was not reisolated from the petioles. The absence of the fungus in these tissues was interesting. Evidently, the fungus did not become established either in the vessels or in parenchyma tissue. While vascular parasites usually colonize vascular tissues predominantly, nevertheless they must penetrate through cortical parenchyma before reaching the vessels. Our general conclusions from all these experiments with young runner plants of strawberry is that they are highly resistant to infection by *Verticillium*. This resistance is maintained when the young plants strike root in, or are transplanted to, recently sterilized soils carrying a high inoculum potential. It is equally effective if the plants are dipped in heavy spore suspensions at transplanting and maintained afterwards under conditions optimal for *Verticillium*.

Study of Relative Pathogenicity of Different Isolates of *Verticillium* to Strawberry

From our inoculation experiments with runner plants it was apparent that these were not suitable for the study of the relative pathogenicity of different isolates of *Verticillium* and the symptomatology of the disease. Therefore, in the following experiments plants grown in the previous growing season and overwintered in the field were obtained from commercial nurseries. Henceforth, these are called "1-year-old plants". From 5000 of such plants a drastic selection was made for uniformity of size and condition of the root system. The diameter of the crowns of selected plants was about 12–15 mm, and the roots were white and healthy. The older leaves were removed, leaving only two or three nearly unfolded leaves, and the root system was trimmed to 4 inches in length. Finally, such plants were thoroughly cleaned and washed. Random culturing of petioles of the detached outer leaves was carried out, and, since no *Verticillium* was isolated, these plants were considered to be free from incipient infection.

Inoculation was performed by root dipping for 24 hours in inoculum and the experiments were carried out under the following three types of environmental conditions:

(i) Under controlled conditions in growth chambers in which the temperature was 22° C, and relative humidity 72%. The length of photoperiod was 14 hours, the intensity of illumination being 1150 foot-candles at the plant surface.

(ii) In a cool and humid atmosphere in a greenhouse. The bench on which the plants were kept was about 4 feet below ground level; as a result, the plants received direct sunlight from above during midday only. During the rest of the day, light was diffused in that part of the greenhouse. The relative humidity of air was always high and frequently in 80–85% range while the temperature range was 10° C to 20° C.

(iii) Under field condition, from 12 June to 25 September, 1959, at the Vineland Horticultural Experiment Station. The details of relevant meteorological data were obtained from the Experiment Station.

A detailed report of the experiments under the three conditions described will now be presented.

Experiment (i): Under Controlled Environment

For this experiment 16 isolates of *Verticillium* (viz. A, B, C, D, E, G, H, I, J, L, N, O, P, S, R, T, and U) and an isolate of *Fusarium* (viz. F) were used for inoculation. Nine plants were inoculated with each isolate by root dipping in macerated inoculum for 24 hours and the inoculated plants were planted in threes in 6-in. pots containing sterilized John Innes soil mixture. Thirty plants were kept as checks. The plants were placed in three growth chambers all of which had the same temperature, relative humidity, and light as described before.

Within a week all inoculated and check plants started to grow in a normal manner. At the end of a month one or two plants, both inoculated and checks, showed slight stunting of the inner leaves but otherwise were normal and vigorous. After 56 days, cultures were made from the petioles of the plants in each treatment. *Verticillium* was reisolated only from a few outer leaves of plants showing doubtful symptoms which were inoculated with isolate B and I. No definite wilt symptoms were observed in the other plants during the 3 months they were under observation.

Even though only a few positive reisolations of the fungus from the petioles were obtained, this was evidence that the fungus was aggressive and suggested that infection might have occurred although symptom expression was suppressed. This would imply that the constant environment which was maintained in the growth chambers was more favorable for the growth of the host than for disease expression. From all the evidence at hand, the experimental environment was essentially optimal for the pathogen.

An alternate interpretation of these results is as follows: The few positive results obtained from the isolation studies come from the petioles of leaves which showed atypical symptoms and in each case the rest of the leaves on the plants from which these came remained healthy. Except in these few abortive cases, therefore, infection failed to take place. Since, from all the evidence available, the experimental environment, with reference to temperature, relative humidity, and light, was essentially optimal for *Verticillium*, the results strongly suggest that the plants were in a non-susceptible condition. If this is so, it could be argued that the environmental conditions supported a development which, in physiological terms, was essentially the same as that of current season runner plants which have been demonstrated to be virtually immune from infection.

Whichever interpretation is correct, the decisive importance of the physiological condition of the strawberry plant on the initiation of infection and the subsequent course of pathogenesis stands out as a factor of hitherto unrecognized importance. This invites further investigation.

Experiment (ii): In Cool and Humid Atmosphere in Greenhouse

In this experiment, 18 isolates of *Verticillium* (A, B, C, D, E, G, H, I, J, L, N, O, P, Q, R, S, T, and U) and an isolate of *Fusarium* (isolate F) were used for inoculation by the same root-dipping method. Thirty plants were

treated with each isolate for 24 hours and then potted in sterilized soil mixture with three plants in each 6-inch pot. Sixty plants were kept as checks. The plants were placed on a bench in the cool and humid portion in the greenhouse in the month of May.

The plants began to grow in a normal way and unfolded new leaves very rapidly. However, the petioles of the leaves in all plants became much longer while the laminae showed slight etiolation, which was apparently due to insufficient light.

Typical wilting did occur in this series and was first observed after 28 days in some of the plants inoculated with the isolates G, B, and I. *Verticillium* was reisolated from all wilted leaves. Within the next 12 days wilting of the outer leaves was noted in the plants treated with all other isolates except U and F. The check plants remained normal. *Verticillium* isolates identical with those used in the original inoculations were recovered from the petioles of all wilted leaves. The two isolates U and F, however, were not reisolated nor was *Verticillium* derived from the petioles of the check plants. No distinctive differences in the symptomatology were apparent among the different treatments and no plants were completely killed. There was evidence, however, of differences in the rapidity of attack by the different isolates. For example, the first wilting was detected in the treatments with isolates G, B, and I on the 28th day, with E and T on the 34th day and with the rest of the isolates (viz. A, C, D, H, J, L, N, O, P, Q, R, and S) on the 40th day after inoculation. The isolates U and F proved to be completely non-pathogenic under these experimental conditions.

These plants were kept under observation for a period of 6 months, from June to November. By the end of 4 months apparently healthy runner plants developed from both infected and check plants. Although not all of the infected plants produced runners, at least three runner plants were available from many of the plants in each inoculated series. This presented an unusually favorable opportunity of investigating whether runner plants contract the disease from infected mother plant through the stolons or independently from soil. From theoretical considerations, if the pathogen proceeds through stolons and only in this manner infects the runner plants, one would expect such runner plants to wilt whether planted in sterilized or infested soil, while the runners from healthy plants should not be infected when planted in sterile soil. On the other hand, if infection takes place only from soil, runner plants from both infected and healthy plants should show disease symptoms only when planted in infested soil. The third possibility is that infection may take place both from infected mother plants and infested soil, in which case the runners from the infected and healthy plants should show the disease in sterilized and infested soil respectively. On the other hand, if the runner plants are highly resistant to infection, no disease symptoms should occur in the runner from infected plants even when planted in infested soil.

To test these postulates several infected and healthy plants which had at least three runners attached to each plant were selected. Sterilized soil was inoculated with all the different isolates in separate flats and the flats were arranged in such a way that, for instance, runners from a mother plant which

had been treated with isolate A could conveniently be trained on the flat of soil inoculated by the same isolate, as well as on a flat containing sterilized soil. That is, runners from a mother plant inoculated with a particular *Verticillium* isolate were planted in sterilized soil and in soil infested with the same isolate. Out of three runners from an infected mother plant two were planted in infested soil and the other in sterilized soil for each isolate. In the same way runners from healthy plants were planted in both infested and sterilized soil.

The runner plants were observed for 2 months after they were set out in the above manner, but no symptoms appeared in any of these plants. Isolations were made from the petioles of all the runner plants but *Verticillium* was never reisolated.

These results support the findings of the earlier infection experiments with young runner plants that these rarely, if ever, contract the disease even from an infected mother plant but are highly resistant in the juvenile condition. Striking evidence is afforded here that in their susceptibility to *Verticillium*, 1-year-old plants and the current year runners are very different.

Experiment (iii): Under Field Conditions

The decision to run an infection experiment under field condition was reached mainly for two reasons. The results of other experiments already recorded indicated that current season runner plants are resistant to infection, as were also 1-year-old plants when forced in a favorable and essentially constant environment. Since it was still impossible to define the physiological conditions which underwrite susceptibility, recourse was had to year-old plants growing in the field, because *Verticillium* wilt is frequently encountered in the Niagara Peninsula under these conditions. Field conditions must accordingly provide also a microbiological balance among the soil flora conducive to *Verticillium* attack. It was hoped, therefore, that field infection studies would not only be valuable in themselves but that they might help interpret the confusing results of the inoculation studies which had been carried on under more controlled conditions.

A plot of land on which strawberries had been growing for the previous 3 years was available for conducting this experiment at the Vineland Horticultural Experiment Station. The use of such a plot had both advantages and disadvantages. One great advantage was that the soil was known to be favorable for strawberry cultivation and for wilt incidence, while the chief disadvantage was that the soil inevitably was infested to some extent by the wilt pathogen. Therefore care had to be taken in the preparation of the field and a soil fumigation was necessary before the experimental plants were set out. The plot was ploughed and harrowed three times by turning the soil over repeatedly to a depth of 1 foot. As far as possible, all the old roots and debris of plant materials were removed. The next important step was careful fumigation of the plot both for *Verticillium* and nematodes. Since Wilhelm and Kock (25) had reported successful results with chloropicrin, this compound was used for fumigation. Fumigation was done by a commercial firm with a power-driven applicator which injected about 2 cc of the chemical at a depth

of 3–4 inches in soil at 6-in. intervals. After fumigation a water seal was given by drenching the plot completely.

Soil samples were collected before fumigation from 40 locations in that plot to check for *Verticillium*, nematodes, and the level of potassium, nitrogen, and phosphorus. The number of parasitic nematodes (*Pratylenchus* sp.) 34 per pound of soil was kindly determined by Mr. J. L. Townshend, Nematologist, at the Plant Pathology Laboratory, St. Catharines, Ont. That the level of K, N, and P in the soil samples was adequate for plant growth was determined form the Plant Nutrition Section, Horticultural Experiment Station. The presence of *Verticillium* in the samples was not detected although a variety of techniques were employed. Fourteen days after fumigation the plot was considered to be ready for planting. Eighteen isolates of *Verticillium* (viz. A, B, C, D, E, G, H, I, J, L, N, O, P, Q, R, S, T, and U), a mixture of all isolates denoted by MX, prepared by mixing equal proportions of the macerated inoculum of each isolate of *Verticillium*, and a species of *Fusarium* (isolate F) were used for inoculation. Sixty plants were inoculated by root-dipping for 24 hours with each inoculum, and 60 plants were kept as checks. Thus in all there were 21 treatments, including the check, which was denoted by the letter K in this experiment. The method of planting was as follows: After the inoculations were made in the laboratory, 60 plants of each treatment were divided into groups of three and were put in plastic bags to avoid contamination during planting. Thus, from 21 treatments, 420 groups of three plants were distributed in the field in such a way that each group was separated from its neighbor by a 4-ft gap, while the plants of each group were planted 2 feet apart. The date of planting was 12 June, 1959. A daily record of temperature was obtained from the meteorological report of the Horticultural Experiment Station. Available soil moisture was maintained around 100% and was measured, as previously indicated, by the use of combination of readings in Bouyoucos moisture and V-O-M meters.

The plants were observed daily, and within 2 weeks most of them were established and produced new leaves. At this time a survey was made to be sure that all the distorted or damaged leaves had been removed from the plants. A record was made also of those plants which did not establish well within 21 days and the final assessment of disease incidence was based upon the number of established plants from that time.

The first wilting was noticed, 33 days after planting, in the outer leaves of a few plants treated wih isolates A, B, C, E, L, M, O, P, and R. The respective fungus isolates were recovered from the petioles of these wilted leaves. Within the next week the disease progressed and certain distinctions among the different treatments were noticeable with respect to the number of plants infected and the rapidity with which certain isolates completely killed a large number of plants. From external symptoms the plants were classified into eight groups and the number of plants belonging to each group was recorded for each treatment. The symptomatological grouping of the plants was as follows:

Group 1. Normal — plants healthy in appearance and comparable to the check plants.

Group 2. Small — plants essentially normal except for being smaller in size.

Group 3. Stunted — plants showing shortening of the petioles of inner leaves and reduction in leaf area. The leaves were dull green, occasionally yellowish-green in color with slight rugosity.

Group 4. Wilted — plants with two to three outer leaves drooping or lying flat on the ground; the color of the laminae changing from greyish-green to brown with a little rolling of leaflets. Finally, the leaves become completely dried and brittle with a stiff petiole. This symptom was observed only in the outer leaves.

Group 5. Stunted–wilted—plants showing stunting of the inner leaves and wilting of outer ones. The reverse situation has never been observed.

Group 6. Wilted–dead—plants died within short time after the outer leaves wilted. No distinct stunting had occurred by the time they died.

Group 7. Stunted–dead—plants severely stunted; eventually died. No wilting of outer leaves was observed. This condition was only rarely encountered.

Group 8. Stunted–wilted–dead—plants, which after being in a stunted–wilted condition for some time, later died. Death ensued slowly as compared to wilted–dead group.

Another small group of plants, which by the end of the season had recovered from an initial mildly infected condition, were included among the normal plants. The progressive changes of symptoms in individual plants and the number of plants within the above-mentioned groups were recorded at four stages. The first, second, third, and fourth readings were taken 45, 53, 60, and 106 days after the date of planting. A lapse of 12 days was allowed between the date of first appearance of symptoms and the date of the first readings, because by that time all the differences in the infection capabilities among the different isolates were apparent in varying degrees. The first three readings were taken at weekly intervals, since the disease progressed rapidly during the 45–60 days after the planting period. The fourth and final reading was deferred until 106 days, because, during the last 46 days the disease progressed slowly. Thus the majority of the plants which were not affected during the first 60 days remained healthy throughout the entire 106 days period, while those which were infected but surviving in a stunted or stunted–wilted condition, remained alive for a long period. Some of the mildly infected plants even recovered from the disease and became normal by the end of the whole period.

The number of plants which belonged to the different symptomatological groups in the four readings was recorded. The results of two contrasting isolates, A and B, are shown in the Tables III and IV. Results of other isolates are omitted in this paper but are available in the original thesis (1).

The final number and percentage of the normal, (total) infected, dead, and infected (not dead) plants are shown below the respective tables. The total number of established plants on which the percentage was calculated is indicated by the total number of plants in the first reading. In each reading following the first one, the number of dead plants which occurred in the previous reading was excluded but is indicated in parenthesis with a minus sign

between the total number of plants in one reading and the previous. The number of plants which recovered from the disease is also put in parenthesis next to the final reading on normal plants, while the total number of plants which remained alive until the end of the season is indicated by subtracting the number of dead plants from the total number of plants in the last reading, next to the number of dead plants.

TABLE III

Summarized results of inoculation with isolate A
(*Verticillium* isolated from apricot)

Symptom group	Readings			
	1st	2nd	3rd	4th
Normal	31	28	28	33(5)
Stunted	7	5	1	1
Wilted	5	3	3	0
Stunted–wilted	1	4	7	4
Wilted–dead	1	3	0	1
Stunted–wilted–dead	0	1	1	0
Total number of plants	45 (−1)	44 (−4)	40 (−1)	39 (−1) = 38

NOTE: The final number and percentage of plants was:
(1) normal 33 = 73.3%
(2) total infected 12 = 26.7%
(3) dead 7 = 16.6%
(4) infected (not dead) 5 = 10.1%

It will be noted in the above table that although stunting, wilting, and even death of some plants occurred by the time the first reading was taken, ultimately the percentage of infection was not high. The disease progressed slowly after the initial attack and there were a few cases of recovery.

TABLE IV

Summarized results of inoculation with isolate B
(*Verticillium dahliae* Kleb, isolated from strawberry, C.B.S.)

Symptom group	Readings			
	1st	2nd	3rd	4th
Normal	6	6	6	6
Stunted	3	0	0	0
Wilted	8	2	1	0
Stunted–wilted	4	5	2	2
Wilted–dead	24	5	1	0
Stunted–wilted–dead	5	3	3	1
Total number of plants	50 (−29)	21 (−8)	13 (−4)	9 (−1) = 8

NOTE: The final number and percentage of plants was:
(1) normal 6 = 12%
(2) total infected 44 = 88%
(3) dead 42 = 84%
(4) infected (not dead) 2 = 4%

In the above table, it will be noted that the first onslaught was tremendous. Characteristically this isolate produced very rapid wilting and death of attacked plants, a large number of which were involved. Most of the infected plants were killed outright.

From the results of this field inoculation experiment the following characteristic aspects may be noted: Essentially the same range of symptom expression was found with all the isolates, although the predominance of either wilting or stunting varied. Rapid killing was invariably more closely associated with wilting than with stunting. Stunted plants in general survived for a much longer period than did wilted ones. Certain isolates behaved in a characteristic way in other respects, on the basis of which smaller groups are recognized among the isolates. These groups are distinguished mainly by the percentage of infection and the rapidity of attack and partially by the characteristic predominant symptoms they incite. For instance, isolates E, B, and C characteristically produced rapid wilting and death of a large number of plants, while isolates I, O, and H produced stunting and a slow killing of plants. The severe stunting incited by isolate I and O was almost diagnostic for these isolates in the later stage of disease development. The isolates P, Q, and R had almost similar effect on the plants and produced wilting with a comparatively slow progress of the disease. Isolates N, L, A, and J, although they produced similar symptoms to those by P, Q, and R, consistently infected a lower percentage of the inoculated plants. Isolates U, G, T, and D were all only very slightly pathogenic with very low percentages of infection.

On the basis of their total pathogenic potentialities, in terms of percentage of infection and the rapidity of the progress of disease caused by them on strawberry plants, the isolates of *Verticillium* could be classified in the following categories:

Category	Isolates	Percentage of infestation
1. Most virulent	E, B, C	Over 80%
2. Virulent	I, O, S, H	58–79%
3. Moderately virulent	P, Q, R	45–56%
4. Slightly virulent	N, L, A, J	20–40%
5. Very slightly virulent	U, G, T, D	0–10%

This classification is obviously more or less arbitrary, and sharp distinctions between the groups will be evident only at the extremes. Considered as a whole, the isolates represent an intergrading series in terms of pathogenic abilities. The series may be represented thus from higher to lower pathogenicity: E, B, C, I, O, S, H, P, Q, R, N, L, A, J, U, G, T, D. The results indicate that the isolates differ in quantitative rather than qualitative characters with respect to their infection capabilities. The pathogenicity of the most and the least virulent of the isolates was so strikingly different, however, that this factor must be recognized as of the utmost importance in the epidemiology of *Verticillium* wilt of strawberries.

During the 106-day period, from June 12 to Sept. 25, 1959, that this experiment was in progress, the range of fluctuation in atmospheric temperature remained fairly constant, except during the first 2 and the last 2 weeks. During the rest of the period, the maximum temperature fluctuated from 21° C to 32° C and the minimum temperature from 15° C to 22° C, with a very few exceptional days when the temperature was below 12° C or above 32° C. Furthermore, the trend in the fluctuation of temperature during the first

60 days and the last 46 days was practically the same. This suggested that the slow progress of the disease during the last 46 days was not influenced by temperature. This conclusion is also supported by the fact that, in all the treatments after the very initial onset of infection, the numbers of normal plants remained practically unchanged throughout the rest of the period, with very few increases or decreases. This fact suggests also that after the experimental inoculations no secondary infections from the soil took place.

Verticillium could be reisolated only from obviously infected plants and, with one exception, it was always the same isolate with which the inoculation was made. The exception was the isolate G, which was a mucoid, non-microsclerotia-producing culture at the time of inoculation but, after reisolation, it became a regular microsclerotia-producing type. The random distribution of all isolates in the field and their reisolation in their original form suggest a very limited spread of the pathogen through soil during a single growing season. This is also evidenced from the observation that normal plants within a distance of 2 feet from a badly infected plant never became infected. This may explain the sporadic and scattered outbreak of the disease in naturally infested soil, since the distribution of the pathogen is certain to be sporadic.

Further Studies of the Disease Status of Current Season Runner Plants Under Field Condition

As occurred also in the greenhouse experiment, the normal and infected plants which survived in the previously described field experiment produced runners in due course. In most such cases there were no symptoms of disease in the runner plants, even where the mother plants later were killed. In only a very few instances were the wilting or stunting of current-year runner plants encountered. Isolations made from apparently healthy runner plants, whether attached to diseased or normal mother plants, were always free from *Verticillium*, whereas in the few instances where *Verticillium* was reisolated from stunted or wilted runner plants these invariably were attached to a badly diseased mother plant. This indicates that, in a small number of cases, infection of young runner plants apparently may occur from an infected mother plant. Since the runners from the apparently normal plants never produced any disease symptom, it is apparent that the infection did not take place from the soil in those cases where definite symptoms of the disease were encountered in the runner plants. However, these exceptional instances of wilted runner plants in no way invalidates the evidence adduced that young strawberry runner plants are highly resistant to *Verticillium*. This conclusion was further supported by the evidence that *Verticillium* was reisolated only from the older outer wilted leaves and never from the very young newly unfolded leaves of infected plants. There is a suggestion, which requires further substantiation, that, in the few instances where infected runner plants were encountered, we were dealing with early infections of the rapidly advancing type at a time when the runners were so completely dependent as to be almost a part of the infected mother plant. If such were the case, however, it is still difficult to explain why the pathogen does not invade the very young leaves of infected plants.

Symptomatology of *Verticillium* Wilt on Pocahontas Variety of Strawberry

After studying the effects of different isolates of *Verticillium* under different environments and obtaining definite evidence that the symptoms expressed are invariably due to infection by *Verticillium*, a discussion of the confused problem of symptomatology seems to be in order.

Under both greenhouse and field conditions, symptoms did not appear until from 28 to 40 days after inoculation. Under a favorable constant environment, their expression was depressed and delayed up to 56 days.

In the early phase of disease development one or two outer leaves show a change of color from normal green to greyish-green accompanied by mild chlorosis in the interveinal tissues of the laminae. Then, browning of veins begins and usually starts from the base and proceeds upwards. This is followed by a slight desiccation of the leaflets, and the drying of the petiole. Brown streaks often develop on the petiole which may extend and girdle the latter completely. However, lighter brown streaks are often found on the petioles of healthy plants which seem to arise from mechanical injuries since from these *Verticillium* has never been isolated. Similar brown streaks may also be found on the stolons, but are not invariably associated with infection. As soon as the petiole becomes somewhat drier and discolored, the whole leaf droops and wilts or lies flat. At this stage, according to the observations in this study, if the wilted leaves be in contact with moist soil necrosis begins from the margin of the leaflets accompanied by a greenish water-soaked area in the center, whereas, if the leaves just hang down without touching moist soil such greenish water-soaked areas with marginal necrosis are never formed. Therefore, this particular symptom can not be taken as diagnostic of *Verticillium* infection. At the base of petioles a dry rot is more typical than wet rot, which again depends on the presence of overmoist soil. As the petiole gradually dries up, it becomes narrow, dark, and stiff. *Verticillium* may be obtained very easily from such wilted petioles of outer leaves. The inner younger leaves never show such wilting. As the disease progresses, the petioles and laminae of newly formed leaves become smaller and smaller, often with mild chlorosis and some rugosity. Thus the plant assumes a stunted form.

When the crown of an infected plant is split into two halves, in most instances a diffused sort of browning or brown spots may be observed in the fleshy portion. No localized browning was evident in the vascular region. The lateral crowns, if developed from an infected plant present no sign of brown discolorations in the fleshy part or in the vascular region. In this connection, it should be mentioned that as soon as a crown is cut into two halves a general browning due to oxidation appears on the cut surface. This browning can be avoided if the freshly cut crown is immediately immersed in 1% ascorbic acid solution, a reducing agent. In this way the original color of the interior portion of a crown can be maintained for a long time. In completely dead plants, the crown becomes dry, brown, and corky inside, but this can not be regarded as a special feature of the disease.

The roots do not change greatly as a result of infection, although the formation of secondary roots from the originally inoculated roots is reduced.

New roots develop as usual from the original or lateral crowns.

The general appearance of a typically infected plant then is somewhat as follows: Some of the outer leaves will be drooping or lying flat and will be greenish-grey to brownish in color. Browning of veins ensues from the base of the laminae, which ultimately will become dry. The central leaves will be smaller than normal and the whole plant have either a prostrate appearance with loosely arranged leaves or becomes severely stunted with a few small leaves at the center. However, in the field, spot diagnosis is very difficult from external symptoms and the surest method of diagnosis is the isolation of *Verticillium* from the petioles of outer leaves. By contrast *Verticillium* has only very rarely been isolated from roots. This may be due, at least in part, to the fact that roots of strawberry plants usually contain many other fungi which may develop faster than *Verticillium*. The symptoms of *Verticillium* wilt of the strawberry as given above fairly agrees with the description of the disease by Thomas (21). Minor variations in symptoms have also been noted by other workers (12, 14, 24).

Infection Experiments on Tomato, Eggplant, and Peach Seedlings with Different Isolates of *Verticillium*

Experiment 1: Relative Pathogenicity to Susceptible and Resistant Tomatoes, Susceptible Eggplant, and Peach Seedlings

From the infection experiments with strawberry plants differences in virulence were apparent among the various isolates of *Verticillium*. To find out whether there were as well any qualitative differences in host range among them, the following hosts were inoculated with all the isolates of *Verticillium* and a species of *Fusarium* from strawberry. As susceptible and resistant varieties of tomato, John Baer and Geneva 11 were used; in eggplants the highly susceptible Black Beauty variety and, in peach, seedlings of the susceptible variety Elberta were used. The same 18 isolates of *Verticillium* and an isolate of *Fusarium* were used for inoculation by the same root-dipping method. Thirty seedlings of John Baer and 20 seedlings of Geneva 11 variety of tomatoes, 20 seedlings of Black Beauty variety of eggplants, and 5 peach seedlings were inoculated with each isolate. All the seedlings were about 4–6 weeks old. Eggplants and tomatoes were transplanted in separate flats and peach seedlings in 10-in. pots containing sterilized soil. In addition to root-dipping, macerated fungus inoculum was also added at the base of the transplanted seedlings. Twenty check plants were kept in all cases and all plants were kept in the greenhouse.

Within 4–6 weeks the eggplants showed wilt symptoms in all the treatments, except those with isolate G, H, L, and the checks. *Verticillium* was reisolated from 100% of the plants in all but the G, H, and L isolates. The peach seedlings did not show any wilt symptom within 5 months in any of the treatments. At that time, when naturally the plants shed their leaves, cultures were made from each plant, but *Verticillium* was not reisolated.

In the Geneva 11 tomato variety, while no symptoms of any sort developed, nevertheless certain of the isolates were recovered from culturing the symptomless plants. These isolates (viz. A, C, E, I, J, L, O, P, Q, R, S, and T) were

designated as aggressive but avirulent to Geneva 11, while other isolates (viz. B, D, H, N, G, U, and F) which were not recovered were termed non-aggressive to Geneva 11.

The results of the inoculation of John Baer tomato were not very clear-cut. Definite wilting was not observed in any of the treatments but varying degrees of stunting and yellowing of leaves were evident. Some of the plants died for some reason other than *Verticillium* infection. Isolations were made from 10 plants in each treatment, which showed that there was no infection with the isolates B, D, N, H, and F.

As the numbers of surviving plants were not enough to make a percentage calculation, inoculation of John Baer variety was repeated with a larger number of plants with slight changes in the method of inoculation. John Baer seeds were sown in the previously infected soil flats. When the seedlings were about 3 inches in height (4 weeks old) the roots were injured by disturbing the soil around the roots by a pair of long forceps and fresh inoculum was added to the base of the plants.

The first definite wilt symptom was evident in the treatments with P and T isolates about 3 weeks after the wounding was made. Other plants showed varying degrees of stunting and yellowing of leaves, which were present in the check plants also.

After another 2 weeks the plants were pulled up from each treatment. The apparently normal plants were separated from the rest, which showed stunting

TABLE V

Summarized results of inoculations on John Baer tomato seedlings with different isolates of *Verticillium* and their reisolations from plants, which showed "stunting and yellowing of leaves" and those which were "apparently normal"

Isolates	"Apparently normal" plants	Plants showing "stunting and yellowing of leaves"	% of plants infected
	*	*	
A	3/20	0/12	9.3
B	1/24	0/15	2.5
C	4/18	4/15	24.2
D	1/12	0/14	3.8
E	1/15	1/25	4.0
G	5/25	7/30	21.8
H	0/22	0/17	0.0
I	4/28	1/26	9.2
J	10/18	5/33	29.4
L	7/31	13/31	32.2
N	0/22	1/28	2.0
O	2/28	9/35	17.6
P	4/10	7/12	50.0
Q	9/19	2/10	28.2
R	0/28	1/7	2.8
S	2/15	6/20	22.8
T	0/29	8/34	15.8
U	0/25	4/14	10.2
Checks	53/431	69/400	14.7

*The numerators indicate the number of plants from which *Verticillium* was reisolated and the denominators denote the total number of plants cultured.

and yellowing of leaves, and each plant was cultured. The percentage of infection was determined by the percentage of reisolation of *Verticillium* from each treatment.

The results are shown in the Table V, where the isolates used for inoculations are shown in the first column; in the second column the denominators indicate the total number of apparently normal plants cultured while the numerators denote the number of normal plants yielding the pathogen on isolation. Similarly, in the third column the denominators indicate the total number of plants showing stunting and yellowing of leaves and the numerators denote the number of such plants yielding the pathogen. The fourth column shows the percentage of plants, including both normal and those showing stunting and yellowing, from which *Verticillium* was reisolated.

The results show that most of the isolates can attack John Baer tomatoes and, as in the case of strawberries, the percentage of infection varies with the different isolates. Accordingly the isolates can be arranged in a series of higher to lower virulence as follows based upon the percentage of infected plants.

P(50%), L(32.2%), J(29.4%), Q(28.2%), C(24.2%), S(22.8%), G(21.8%), O(17.6%), T(15.6%), U(10.2%), A(9.3%), I(9.2%), E(4.0%), D(3.8%), R(2.8%), B(2.5%), N(2.0%), and H(0.0%).

Here one finds that the highest percentage of infection is 50% and the lowest is 2.0%–0.%. Therefore, the isolates which caused more than 40% infection were arbitrarily classified as "virulent" to John Baer, e.g. isolate P; those which caused 20–40% infection were classed as "moderately virulent"; those with 10–20% infection capabilities were a "slightly virulent group", while those which produced less than 10% infection were classed as "very slightly virulent".

From this experiment, it becomes apparent that "apparently normal" tomato plants may carry the pathogen without showing any symptom, while plants showing yellowing of leaves or stunting may not have the fungus within them. According to the data obtained in this experiment, out of a total of 831 established plants 52.1% were "apparently normal", and 47.9% showed different degrees of stunting and yellowing of leaves; 12.7% of the normal plants carried the pathogen, whereas only 16.7% of the plants showing yellowing and stunting were infected. Therefore, with John Baer tomato seedlings stunting and yellowing of leaves can not be considered as safe criteria of *Verticillium* infection. However, from definitely wilted plants *Verticillium* has been reisolated in all cases.

Experiment 2: Pathogenicity Test of 3-Year-Old Cultures on Three Varieties of Tomato, viz. John Baer, Bonny Best, and Geneva 11

It has been mentioned previously that the isolates A, C, E, J, L, P_p, and S were viable on a dried agar medium for 3 years. These isolates, along with their corresponding recent isolates, with which all the inoculations in the previous experiments were made, were inoculated to John Baer, Bonny Best, and Geneva 11 tomatoes. Ten seedlings of each variety were inoculated with each isolate by root-dipping and the plants were transplanted into 5-in. pots containing sterilized soil with five plants in each pot.

Within 2 months, definite wilting was not observed in any of the plants but the respective isolates of *Verticillium* were reisolated from each treatment. This showed that the aggressiveness of the isolates was not lost by the older isolates, and the expression of symptoms on both Bonny Best and John Baer were not different or clear-cut. Geneva 11 was, as usual, a symptomless carrier with respect to the isolates tested.

A Tentative Key for the Separation of Different Isolates of *Verticillium* Studied

Considering all the experiments carried out with the different isolates of *Verticillium* and by combining the cultural, morphological, and phytopathogenic characters an attempt was made to separate these isolates. The results are presented in the following key:

a_1. Culture black with abundant microsclerotia ..... B, C, E, J, L, P, Q, R
  b_1. Most virulent on strawberry; % infection over 80% ..... E, B, C
    c_1. Aggressive to Geneva 11 tomato ..... E, C
      d_1. Moderately virulent to John Baer tomato C
      d_2. Very slightly virulent to John Baer ..... E
    c_2. Not aggressive to Geneva 11 ..... B
  b_2. Moderately virulent to strawberry ..... P, Q, R
    c_1. Virulent to John Baer ..... P
    c_2. Moderately virulent to John Baer ..... Q
    c_3. Very slightly virulent to John Baer ..... R
  b_3. Slightly virulent to strawberry ..... J, L
    c_1. Virulent to Black Beauty eggplant ..... J
    c_2. Avirulent to Black Beauty L
a_2. Cultures greyish white with scanty microsclerotia ..... A, H, I, O, N, S
  b_1. Virulent to strawberry ..... I, O, H, S
    c_1. Aggressive to Geneva 11 ..... I, O, S
      d_1. Moderately virulent to John Baer ..... S
      d_2. Slightly virulent to John Baer ..... O
      d_3. Very slightly virulent to John Baer I
    c_2. Not aggressive to Geneva 11 ..... H
  b_2. Slightly virulent to strawberry ..... A, N
    c_1. Aggressive to Geneva 11 ..... A
    c_2. Not aggressive to Geneva 11 ..... N
a_3. Cultures completely white with fluffy aerial mycelium ..... U, T, D
  b_1. Aggressive to Geneva 11 ..... T
  b_2. Not aggressive to Geneva 11 ..... U, D
    c_1. Able to produce microsclerotia on potato plugs ..... U
    c_2. Not able to produce microsclerotia on potato plugs ..... D
a_4. Culture mucoid in the beginning but producing normal hyphae and microsclerotia on passage through strawberry ..... G

Discussion and Summary

With respect to the taxonomy of the causal fungus there is still some difference of opinion as to whether one rather variable species, *Verticillium albo-atrum* is involved or whether this should be resolved into two or more species, largely on the basis of the absence or presence of resting bodies and their type when produced. The earlier concept of an extremely broad host range with very little specialization in parasitism seems still widely held, although more specialized races have recently been reported in a limited number of instances centering around such hosts as peppermint (16), brussels sprout (10), and pepper (11). Optimum conditions for disease development have been

defined largely in terms of relatively high soil temperature and soil moistures above the optimum moisture content for the soil in question, with little evidence that the disposition of the host is of any marked significance. While *Verticillium* wilt frequently occurs as sharp local outbreaks its epidemiology is not well understood. Although the fungus has been shown to be dispersed widely and to considerable depths in some soils, its behavior in soil suggests that it is a root invader rather than a soil inhabitant. Against this general background a special study extending over a period of 3 years has been made of *Verticillium* wilt of strawberries, as it occurs in the Niagara Peninsula, Ontario.

Initially a large number of *Verticillium* isolates was assembled. Many of these came from strawberries and several other hosts from the Niagara area but these were supplemented by named isolates from such respectable herbaria as the American Type Culture Collection, Washington, D.C.; Centraalbureau voor Schimmelcultures, Holland; and Plant Research Institute, Ottawa.

Eighteen isolates were compared in microscopic and cultural characters on a series of culture media. In microscopic characters, in terms of conidiophore and conidial morphology, there was a considerable range of variation but since each isolate proved to have a marked potential for such variation, it was impossible to subdivide them on these characters. In cultural characteristics, the isolates ranged from completely white, through varying intensities of grey to jet black in color. At the extremes they were strikingly different. Howevér, the whole 18 isolates considered as a group merged so gradually that they were inseparable. Under these circumstances it seems to the writer wiser to regard all the isolates as belonging to the originally described species, *Verticillium albo-atrum* R. and B.

Multiple plantings of individual isolates on the same plate exhibited no aversion on the media used. Nor was any antagonism evident between isolates grown in many different associations on the same media. Some isolates on continued culturing lost the capacity of producing microsclerotia. This was regained in some instances on certain media and in culture association with microsclerotia-producing isolates. In this case we are obviously dealing with nutritional requirements and deficiencies rather than with innate capacity for microsclerotia production. This explains in part why microsclerotia production has proved so unsatisfactory as a primary basis of species separation.

Extensive infection studies were carried out with some or all of those isolates of *Verticillium* on strawberry plants under a wide range of experimental conditions. From these a number of interesting new findings emerged and many earlier ones were confirmed.

Of great interest and potential importance was the demonstration that the current year runner plants of strawberries are virtually immune from *Verticillium* infection. This resistance was expressed even when the runners remained attached to the mother plants and were allowed to root in soil heavily infested with *Verticillium*, or in sterile soil with subsequent transplanting, after the roots were dipped in a spore suspension, into either sterile or infested soil. After current season runners have become dormant and overwintered under normal conditions, they break into rapid vegetative development followed by flowering and by this time they have become susceptible. However, in some experiments spring-dug runner plants seemed to remain in

a resistant state when transferred to a controlled environment chambers where rapid growth occurred and conditions were apparently optimal for *Verticillium* attack. The importance of the physiological condition of the host in relation to the susceptibility to *Verticillium* seems to have been hitherto unrecognized and offers a promising field for further research.

There were striking differences among the 18 isolates tested in the virulence of their attack on Pocahontas strawberries but to none of these isolates were they immune. These quantitative difference in pathogenicity ranged from very low virulence, where the attack was exceedingly mild and involved only a small percentage of the population, to extreme virulence characterized by the early onset and rapid development of wilting and the death of virtually the whole population. As the virulence of the isolates decreased, the incubation period tended to be longer, the symptoms comprised progressively less acute wilting and stunting, and the infected plants lived for extended periods before being killed. Virulence seemed to be essentially a function of aggressiveness as well as of pathogenicity, since with decreasing virulence a smaller percentage of the inoculated plants became infected. The etiology of pathogenesis should be amenable to experimental sudy and it would be interesting to investigate the pectic enzyme mechanism of the most virulent isolates and the possibility that their pectin degradation products might induce typical wilt symptoms in strawberry plants.

The pathogenicity of the *Verticillium* isolates which differed with respect to the strawberry was tested with respect to other *Verticillium* hosts, viz., tomato, eggplant, and peach seedlings. On the susceptible tomato variety John Baer, the symptom expression was generally atypical and confusing but significant differences among the isolates emerged in terms of the relative percentages of infected plants from which they could be reisolated in culture. The *Verticillium*-resistant tomato variety, Geneva 11, while it appeared immune in terms of symptom expression, proved to be a symptomless carrier infected with 12 isolates, whose pathogenic potential accordingly must be different from the other six. With the exception of two or three isolates, those that were capable of invading the highly resistant Geneva 11 were also among the more virulent isolates on the susceptible variety, John Baer. On the contrary, some of the isolates avirulent to the resistant Geneva 11 were among the most virulent on strawberry, while isolate T was avirulent on strawberry but was able to attack Geneva 11. The general impression, however, is that the order of virulence against a compatible and a resistant host are fairly closely correlated.

From the entirety of our infection experiments we have a clear demonstration of the existence of physiologic races of *Verticillium*. These races have, for the most part, been able to infect all the limited host range on which they have been tested. On one or more of these hosts, however, they have been characterized by striking quantitative differences in the relative intensity of their attack. On the strawberry they range from lethal to virtually avirulent. Some of the strains which were most virulent on strawberry were isolated from other hosts such as eggplants, while some of the avirulent strains came from the strawberry.

This gives us a much better perspective on the *Verticillium* wilt of strawberry. The intensity of attack is, in the first instance, a function of the virulence of the races locally available and, since these races are not confined to strawberry but attack also some of the commonly occurring *Verticillium* hosts, strawberry rotations have to be designed to avoid such hosts as tomatoes, eggplants, potatoes, and pepper. While this unquestionably complicates the problem immeasurably, other results of our infection studies on strawberries greatly simplified it. This is especially true of the demonstration that current-year runner plants are virtually immune from infection and that normal-appearing, vigorous overwintered strawberry plants do not carry a latent infection. Because of these characters the grower has to give due consideration to the source from which he buys plants but he need not worry over the likelihood of a rapid extension in a first year stand from even a generous scattering of killed mother plants, since, if they have managed to produce established runner plants before being killed, these are almost certain to remain healthy throughout the rest of the season. This prognosis is supported further by the results, which indicate that relatively high inoculum potential is required for appreciable infection and that the fungus moves very little within a strawberry row during one growing season. The development of *Verticillium* wilt in the first fruiting year should now be equally intensively studied.

The symptomatology of *Verticillium* infection on strawberry, tomato, and eggplant has been critically studied. A syndrome has been described which permits reasonably certain diagnosis of the most frequently encountered host response in the different cases. However, with avirulent strains under optimum conditions for disease incidence and with more virulent ones under unfavorable conditions, the symptoms become less and less diagnostic and ever more readily confused with root malfunctions in general. This frequently becomes a limiting factor in assaying the results of infection studies of any sort. Fortunately this difficulty is resolved by our demonstration that a routine isolation of hypochlorite disinfected petioles, stems, cotyledons, or hypocotyls on water agar gives consistent and dependable results. It is so feasible to execute that it should be regularly included wherever possible in assaying *Verticillium* infections.

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STUDIES ON FUSARIUM WILT OF BANANAS

VII. FIELD CONTROL[1]

R. H. Stover, R. C. Hildreth, and N. C. Thornton

Abstract

Flood fallow and silting were the only practices that consistently reduced incidence of banana wilt induced by *F. oxysporum* f. *cubense* (E. F. S.) Snyd. and Hans. The efficiency of flood fallow was greatly increased by interflood plowing, and in most locations by postflood fungicide drenches. Even these treatments, however, did not insure economic control in some areas. It was necessary, therefore, to select areas where soil factors tended to retard rapid disease development. Also, the absence of the banana host for a number of years previous to flood fallow appeared to influence treatment success.

Introduction

Attempts to control *Fusarium* wilt (Panama disease) of 'Gros Michel' bananas outside of Central America have involved: (1) eradication and quarantine; (2) cultivation of existing resistant varieties; and (3) breeding new varieties. In addition Wardlaw (11) discusses the importance of improving soil conditions by drainage, aeration, and maintenance of organic matter, although there was little or no experimental evidence to indicate how effective these procedures might be in reducing disease incidence. Only the use of resistant varieties proved successful. In 1950 Rishbeth and Naylor (4) attempted control by soil fumigation, and addition of organic amendments to the soil. They also studied the influence of fertilizers on disease incidence. In general, results were negative or of questionable importance because of plot size and experimental design. Butler (1) was unable to detect any influence of fertilizers on *Fusarium* wilt incidence in numerous experiments in Honduras extending over a 15-year period.

In Central America, before flood fallow methods were introduced in 1940, measures to cope with *Fusarium* wilt involved new land selection, silting, and attempts to select disease-free propagating material. Studies were also undertaken to determine factors that influenced disease incidence: soil texture, mineral nutrition, pH, irrigation, cultivation, and fertilizer practices (2, 3, 10). The first important outcome of these studies was the observation that on certain soils, economical production could continue for 10 to 20 years, whereas on other soils, particularly those of low pH and light variable textures, production would cease within 2 to 5 years (10). Thus, wherever possible, those soil factors that resulted in low disease incidence were considered in planting new areas even though the exact nature of the factors remained unknown. A second important observation was that infested lands could be made productive again after having a layer of silt from 1 to 3 ft deep deposited by rivers in flood stage. This usually required at least 5 years and as long as 15 years to

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accomplish, as silt depositions were made only during the 30 to 40 days per year when the rivers carried a sufficient volume of water and silt. A large proportion of infested soils could not be silted, however, because of distance from rivers and high elevation. Also the time required for silting depended on rainfall and river flow, which fluctuate greatly from year to year.

Addition of lime and large quantities of organic matter to an infested soil was reported to have yielded good results in one small area of Honduras (5). However, there was no plot replication or repetition of this experiment. Addition of large quantities of lime in other areas of Central America failed to slow disease development (unpublished reports).

Since 1940, *Fusarium* wilt control has involved flood fallow, and more recently, increased emphasis on the use of 'improved' and 'certified' disease-free propagating material. Deep plowing to a depth of 30 inches yielded variable and, in general, unsatisfactory results. Selected areas, where the bananas were formerly destroyed by disease, have been replanted with some success after 3 to 10 years of brush and grass fallow. Again advantage was being taken of unknown soil factors present in areas that hindered rapid disease development. When replanted, these 'resistant' areas would yield 3 to 5 years of economic production whereas most other areas, even after 10 years of brush fallow, were rapidly devastated by disease when replanted. In general, brush fallowing has been of value only in certain selected areas.

Flood fallow was developed rapidly from 1945 to 1955 in Honduras and Panama with mostly favorable results until the second cycle of fallowing was attempted. Flood fallow for periods of 4 to 6 months was undertaken on former banana farms most of which had become unproductive because of disease from 3 to 15 years previously. Flood fallow was considered successful when 3 to 5 years of production was obtained after flood fallow and before loss of about one-half or more of the area to the disease. The plants that survived were chopped down and the area was subjected to a second cycle of flood fallow. Although some failures occurred with the first fallow cycle, resulting in only about 2 years of production before disease was again rampant, they were relatively infrequent when contrasted with failures after the second cycle of fallowing. The reason for this increase in failures has been discussed previously (9). It was apparent that some *Fusarium* was surviving in the surface layer of submerged soil and modifications of the flood fallow procedure were necessary to eliminate these survivors and to increase the efficiency of disease control (7, 9). This was attempted in three ways: (1) manipulation of the surface soil layer by moldboard plowing after flooding, followed by another flood period; (2) fungicide drenches after floodings; (3) production of green manure or other crops after flooding, and other treatments to assist in restoring a soil flora that might be inhibitory to *Fusarium* development. These studies began in 1955 and the first results of some representative experiments are reported in this paper.

General Methods

Experiments testing the effects of interflood plowing, postflood fungicide drenches, and postflood crop amendments were undertaken in different locations in Honduras and Panama. All areas had been flood fallowed previously

and were selected on the basis of rapid disease development following this treatment alone. Numerous clumps of banana plants with *Fusarium* wilt were scattered throughout the areas previous to the experimental floodings. Banana plants and brush were cut down, and the area was thoroughly disked before being inundated with from 1 to 5 feet of water (Fig. 1). After approximately 3 months of flooding, the land was drained and certain areas plowed to a depth of 6 to 10 inches with a moldboard plow. Particular care was taken to see that the surface 1 to 2 inches of soil, silt, and organic matter was turned under. The area was again flooded for about 60 days and then drained, followed by a second plowing in some areas. The second plowing was followed by a final 60-day flooding period, after which the area was drained for further treatments and for planting. The periods between flooding were from 1 to 2 months.

Where fungicides were tested, these were applied after the final flooding, utilizing a portable sprinkler irrigation system. As soon as the soil was sufficiently dry, the plots were rotovated to a depth of 3 to 6 inches. The soil was then sprinkler-irrigated for 20 to 30 minutes with water, followed by 60 minutes of water plus a fungicide, and finally with 10 to 20 minutes of water. The irrigation pump had a capacity of 875 gallons per minute at 50 p.s.i. Efforts were made to wet the soil thoroughly to a depth of 4 to 6 inches with approximately 3/4 inch of water. It required from 1 to 2 months after the experimental area was drained to complete fungicide applications.

Approximately 1 month after all fungicide treatments were completed, the entire area was planted with 'Gros Michel' bananas utilizing carefully selected and inspected planting stock to avoid introduction of disease. Bananas were usually planted 11 or 12 feet apart in a square pattern with approximately 303 to 360 plants per acre. Randomized complete block designs were used throughout. Unplanted borders of at least 20 feet were left around all plots except in experiments 1 and 3. The first cases of *Fusarium* wilt began to appear about 4 to 6 months after planting. Surveys were usually made monthly and all diseased plants were recorded on maps.

Results

Experiment 1

The experimental area was flooded for 90 days and then drained; certain plots were then plowed, and a final flooding of 60 days followed for all plots. After the final flooding, fungicide drenches were applied. There were five replicate plowed plots with 567 plants per plot. Fungicide trials were conducted separately and consisted of three replicate plots with 253 plants per plot and two controls (flood fallow only) per block. Vapam was applied at 75 gallons and Mylone at 250 lb per acre. Calcium cyanamid and sulphur were broadcast between floodings on two plots each at the rate of 3 tons per acre and thoroughly disked into the upper 4–6 inches of soil.

Although all treatments except sulphur resulted in significantly less disease, disease increased at a rate that would preclude economic production (Table I, Fig. 2). About 400 days after planting, the percentage of plants that had yielded a diseased shoot were as follows: flood fallow alone, 87 and 90; flood fallow plus plowing, 52; and flood fallow plus fungicides, 34. However, many

of the shoots in the diseased plants of the plowed and fungicide treatments remained healthy and produced a stem of fruit whereas most shoots in diseased plants in the flood fallow treatment succumbed to disease. For example, 65 and 77% of the check plants (flood fallow alone) did not produce a stem of fruit, whereas 27 and 13% of plants in the plowed or fungicide-treated plots respectively failed to produce a stem of fruit by 400 days after planting. Thus much more total fruit production was obtained as a result of the treatments than the percentage of diseased plants would indicate.

TABLE I

Mean percentage of disease incidence, transformed to angles (6), of flood fallow alone versus flood fallow plus interflood plowing, or postflood fungicide drenches

Days after planting	Plowing trials		Fungicide trials		
	Check (flood fallow alone)	Plowed between floodings	Check (flood fallow alone)	Flood fallow plus:	
				Vapam	Mylone
135	5.5	2.97	2.42	1.21	0.0
150	9.34	3.99	4.71	1.21	1.70
183	15.47	6.09	14.50	4.84	3.92
195	16.52	6.42	15.20	5.63	4.81
218	26.01	11.23	27.23	10.63	9.30
237	30.51	13.94	33.08	13.27	13.29
264	36.91	18.66	38.29	17.34	17.09
294	40.08	21.92	40.10	19.66	18.96
316	41.39	24.09	41.72	22.41	21.55
390	47.61	37.12	46.88	35.10	35.20
Treatment means averaged over all dates	26.94 (20.5)*	14.64 (6.4)	26.71 (20.2)	13.13 (5.2)	12.58 (4.8)
L.S.D. .05	5.84		9.20		
L.S.D. .01	9.68		—		
Regression coefficient	.1786	.1360	.1921	.1350	.1367
L.S.D. .05	.0128		.0195†		
L.S.D. .01	.0169		.0262†		
Coefficient of variation	12%		17%		

*Numbers in parenthesis are mean percentage diseased mats, untransformed from angles.
†L.S.D. between check and one of treatments.

The sulphur and calcium cyanamid plots were of an exploratory nature and not designed for statistical analysis. Comparison with adjacent non-treated areas indicated no reduction in disease incidence as a result of sulphur application. With calcium cyanamid, however, rate of disease development was markedly slower than in adjacent non-treated portions (Fig. 2).

Experiment 2

This area was flooded for separate periods of 3, 2, 2, and 2 months with one or three plowings or one disking between floodings. There were six replicates per treatment with 462 plants per plot.

PLATE I

PLATE I

FIG. 1. Aerial view of flood fallow area. Note relatively solid stands of recently planted bananas adjacent to river, and in upper portion of photo, older areas decimated by disease with a few surviving patches.

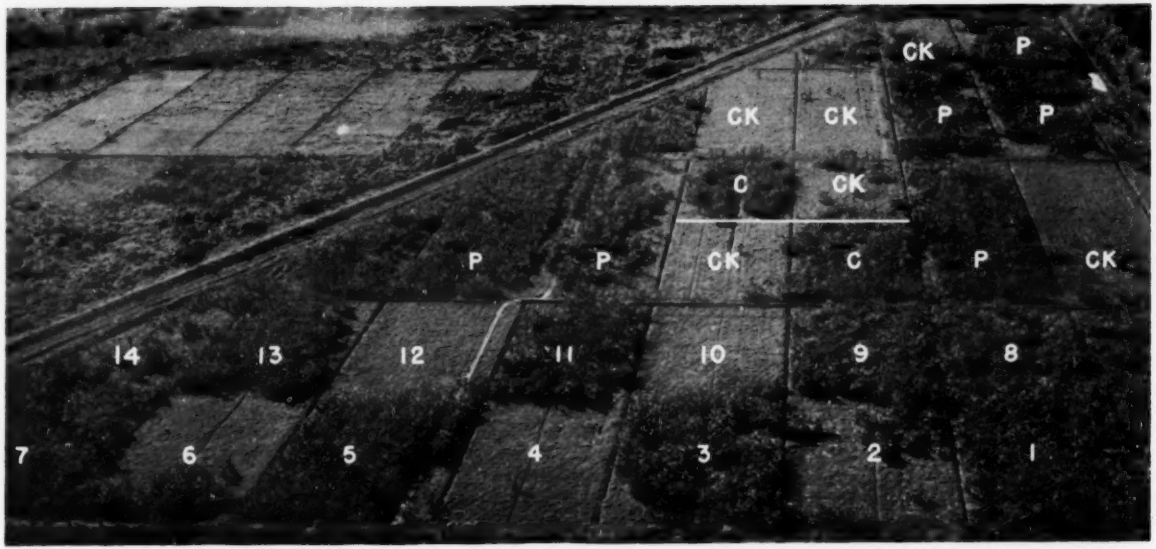


FIG. 2. Aerial view of various treatments in experiment 1 one year after planting. Heavily diseased areas were cut down as they were no longer productive. Treatments were as follows: 1, 11, 13, Vapam 75 gal/acre; 3, 5, 7, 9, Mylone 250 lb/acre; 2, 4, 6, 8, 10, 12, 14, checks for fungicide trials; P—plowed, C—cyanamid, and *ck*—checks (flood fallow only) for plowing trials. Note encroachment of disease around borders of treatments from untreated plots.

The slowest rate of disease development was in the treatment plowed once between each of four floodings, or a total of three plowings (Table II, Figs. 3 and 4). The L. S. D. of the regression coefficients indicates that each treatment is different from the others. Even allowing for the healthy development

TABLE II

Mean percentage disease incidence, transformed to angles (6) of flood fallow alone versus flood fallow and interflood disking or plowing

Days after planting	Check (flood fallow alone)	Disked once between floodings	Plowed once between floodings	Plowed thrice between floodings
121	7.10	1.40	0.45	0.45
190	18.57	8.86	5.39	3.14
210	23.94	12.17	8.39	4.88
240	38.11	19.04	15.20	8.49
289	49.78	26.56	22.18	13.44
331	64.32	34.89	34.02	20.67
378	71.27	42.75	38.20	26.94
427	77.54	47.28	41.64	30.80
471	90.00	67.31	44.90	34.16
Treatment means averaged over all dates	48.96 (56.9)*	28.92 (23.4)	23.37 (15.7)	15.88 (7.5)
L.S.D. .05	11.41			
L.S.D. .01	15.77			
Regression coefficient	.2459	.1795	.1426	.1078
L.S.D. .05	.0259			
L.S.D. .01	.0343			
Coefficient of variation	25%			

*Numbers in parenthesis are mean percentage diseased mats, untransformed from angles.

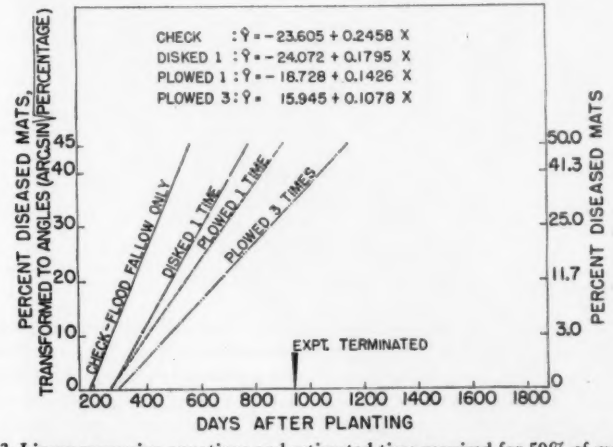


FIG. 3. Linear regression equations and estimated time required for 50% of mats to be diseased in flood fallow versus flood fallow plus interflood disking or plowing trials.

of some shoots from diseased plants in the treatment with the lowest disease incidence, it is apparent that economic production would not exceed 2½ to 3 years from time of planting.

Experiment 3

This area was flooded for separate periods of 3, 2, and 2 months with plowing between floodings. Also, calcium cyanamid was applied at the rate of 3 tons per acre to one block of 2 acres between the second and third floodings. Plots to be treated with fungicides were lightly disked between floodings. After the final flooding, fungicides were applied at the following gallonage per acre: formaldehyde, 250; Vapam, 50; allyl alcohol, 40. There were four replicates per treatment and two checks (flood fallow only) per block with 294 plants per plot.

All treatments greatly reduced disease incidence. Disease development in the Vapam plots was slower than among the other treatments (Table III and Fig. 5). Although no statistical analysis of the calcium cyanamid treatment is possible because of lack of replication, the pattern of disease incidence in the surrounding area indicates that cyanamid reduced rate of disease development (Fig. 6). In comparison with the previous experiments, disease development has been much slower in all treatments and an economically productive life of from 3 to 5 years can be expected.

TABLE III

Mean percentage disease incidence, transformed to angles (6), of flood fallow alone versus flood fallow and interflood plowing or postflood fungicide drenches

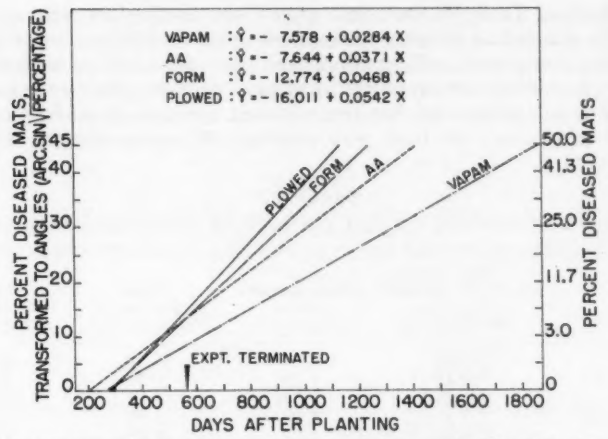
Days after planting	Check (flood fallow alone)	Allyl alcohol	Vapam	Formal-dehyde	Plowed twice	L.S.D.* .05	L.S.D. .01	Coefficient of variation (%)
305	22.96	3.20	1.18	1.67	0	7.89	11.06	57
338	25.89	4.55	1.18	1.67	1.18	8.46	11.86	55
376	36.53	6.40	3.20	5.01	4.57	11.03	15.47	48
406	50.90	8.04	4.55	7.66	7.33	15.92	22.32	49
462	52.96	10.08	6.02	8.93	10.10	15.61	21.89	45
511	55.24	11.72	6.97	11.05	12.18	15.09	21.16	40
566	56.78	12.46	8.03	13.32	13.20	13.99	19.61	36
Regression coefficient		0.0371	0.0284	0.0468	0.0542	0.0133		
Prediction to 50% infection (years)		3.9	5.1	3.4	3.1			

*L.S.D. for comparing treatment with check.

About 12 months after planting, the high rate of disease development in the check plots leveled off and disease development in the treatments continued at a very slow rate. Also some plants previously showing diseased shoots subsequently yielded healthy shoots that produced fruit. Therefore, actual percentage of plants diseased over the period of the experiment became of less importance than number of healthy-appearing plants at the time of individual

PLATE III

FIG. 4. Aerial view of non-plowed (*ck*) and plowed plots (P) approximately 10 months after planting in experiment 2. No diseased plants have been cut down.


VAPAM : ŷ = - 7.578 + 0.0284 X
AA : ŷ = - 7.644 + 0.0371 X
FORM : ŷ = - 12.774 + 0.0468 X
PLOWED : ŷ = - 16.011 + 0.0542 X
PERCENT DISEASED MATS, TRANSFORMED TO ANGLES (ARC SIN √PERCENTAGE)
PERCENT DISEASED MATS
PLOWED
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DAYS AFTER PLANTING

FIG. 5. Linear regression equations and estimated time required for 50% of mats to be diseased in flood fallow plus interflood plowing or postflood fungicide drenches.

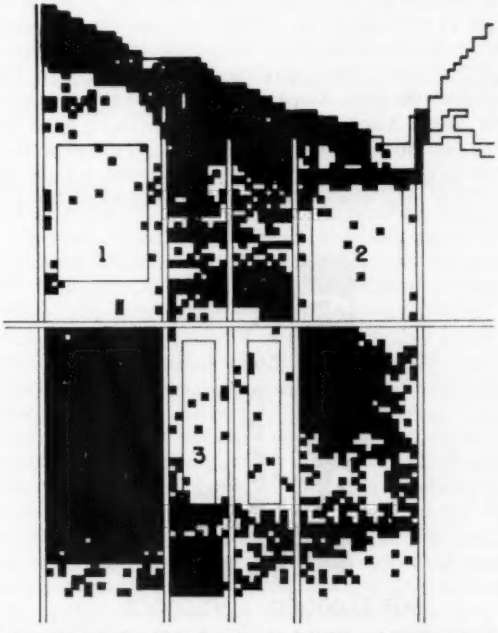

FIG. 6. Chart showing location of diseased mats 511 days after planting in blocks treated with: (1) Vapam 50 gal/acre (2) allyl alcohol 40 gal/acre, and (3) 3 tons cyanamid per acre; surrounding areas were non-treated.

surveys. Data in Table IV show that even in the check plots, where a total of 70% of the plants had shown a diseased shoot up to 566 days after planting, 50% of the plants were still standing, and only 8% of these showed visible disease at the survey 628 days after planting. Therefore, the number of plants destroyed or non-productive because repeated appearance of diseased shoots precluded production of fruit was another indication of rate of disease development.

TABLE IV

Percentage of mats diseased, destroyed, and healthy at various intervals after planting

	Total mats showing a diseased shoot up to 566 days (%)	Mats diseased only at 628 days (%)	Mats destroyed at 628 days (%)	Mats healthy at 628 days (%)
Check	70.00	8.13	50.34	41.49
Allyl alcohol	6.45	1.29	1.29	97.45
Vapam	1.95	0.34	1.12	98.57
Formaldehyde	5.31	2.99	0.78	96.26
Plowed	5.21	1.87	0.95	97.21

Experiment 4

This area was flooded for 3, 2, and 2 months with plowing as a treatment between floodings. A portion of the plowed and non-plowed areas was planted with sorghum after the final flooding. The sorghum (30 tons green weight per acre) was disked 2 months after planting, allowed to dry on the soil surface, and then plowed into the soil. 'Gros Michel' bananas were planted approximately 1 month later. There were nine replicates of each treatment containing 126 plants per plot. An area contiguous with the experimental area and with the same history was left unflooded and plowed a total of 4 times once every 3 months. This area was divided into nine plots of 126 plants each and

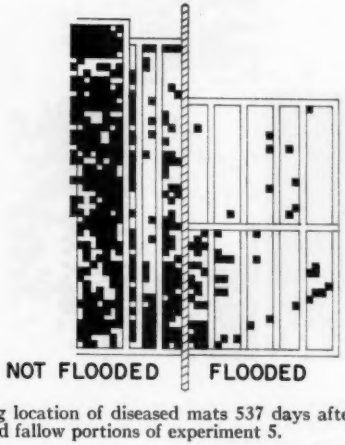

FIG. 7. Chart showing location of diseased mats 537 days after planting in non-flood fallow and adjacent flood fallow portions of experiment 5.

planted at the same time as the flooded area. Although no statistical comparison can be made with data from the flooded area because of lack of randomization, results from the non-flooded block indicate the great effect of flooding in reducing disease incidence (Fig. 7, Table V). Plowing between floodings significantly reduced disease but there was no effect attributable to one cropping with sorghum (Table V).

TABLE V

Effect of interflood fallow plowing and postflood fallow sorghum on incidence of *Fusarium* wilt

	Treatment means per plot		
	Sorghum	No sorghum	Average
Plowed	3.2 (2.55)*	1.2 (0.96)	2.2
Not plowed	13.2 (10.49)	11.1 (8.81)	12.2
Average	8.2	6.2	

	Analysis of variance		
		F required for significance	
	Calculated *F*	.05	.01
Plowed vs. not plowed	9.38	4.26	7.82
Sorghum vs. no sorghum	0.40	4.26	7.82

*Total plants diseased to 568 days after planting averaged for nine replicate plots per treatment with 126 plants per plot. Numbers in parenthesis indicate percentage plants diseased; an adjacent non-flood fallowed plowed area was 77% diseased 537 days after planting.

Discussion

Once an area was heavily infested with *F. oxysporum* f. *cubense*, a substantial reduction in population of the pathogen, as indicated by disease incidence on 'Gros Michel' bananas, could be obtained by silting or flood fallow. Even when bananas were not present for 10 years or more, and the area was in brush, pasture, or sugar cane, the pathogen was still capable of causing rapid disease development when the susceptible host was re-established. Unsuccessful attempts had been made previously in non-flood fallow areas to reduce the pathogen to a level that would result in an economic crop by fungicide drenches, urea applications of up to 6 tons per acre, and soil fumigation with methyl bromide or chloropicrin. The success of flood fallowing depended on the pathogen's failure to survive in depth in submerged soil. However, it did survive in the surface soil layer and in the water (7, 9). Postflood fungicide drenches and interflood plowings have reduced this surviving population and increased the efficiency of flood fallowing as indicated by lowered disease incidence. Nevertheless, even these treatments have not always insured an economic return from this susceptible perennial crop that must yield for at least 4 to 5 years after planting to be profitable. For such relatively long periods of high yield the pathogen population must initially be at a low level (9). Also, disease must develop at a slow rate for at least the first 2 years after planting before the disease incidence curve begins to swing sharply upward.

Disease development is dependent not only on the initial inoculum potential but also on soil factors that hinder or favor pathogen survival and infection (8, 9). Wide variation in rate of disease development among different areas has been characteristic of flood fallow history, indicating that unknown soil factors are variable from one area to another and are exerting considerable influence. Therefore, in addition to reducing the pathogen population by the treatments described, advantage can be taken of natural soil factors that are less favorable to disease development. Thus for second-cycle flood fallow on a commercial scale, only those areas would be selected where previous disease history indicated that some natural factors are present that tend to hinder a rapid rate of disease development. In addition, efforts are now being made to destroy the remaining banana plants after an area is no longer productive so that the inoculum is gradually lowered over a period of several years before attempting rehabilitation. With these combinations of: (1) selection of area based on previous slow-disease development; (2) lowering the initial amount of inoculum by removing the host; and (3) subjecting the remaining inoculum to flooding, plowing, and (or) fungicides, disease incidence could be reduced to a low level in selected areas.

The usefulness of a grass or crop fallow for a period after flood fallow remains to be determined. There is no evidence in the data reported above that cropping with sorghum was helpful in reducing disease incidence. Studies are continuing with other crops and longer rotations.

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SEED DEVELOPMENT IN ADHATODA VASICA NEES[1]

H. Y. Mohan Ram

Abstract

The events following fertilization up to the maturation of the seed in *Adhatoda vasica* have been described. The first two transverse divisions accompanying the division of the primary endosperm nucleus partition the embryo sac into (1) a micropylar chamber, (2) a chalazal chamber, and (3) a central endosperm chamber. The first two develop into binucleate haustorial structures, while the bulk of the endosperm is derived from the products of the central endosperm chamber. An unusual case of a micropylar haustorium containing four hypertrophied nuclei and four small nuclei has been observed. The central endosperm chamber is partly cellular and bears a tubular free nuclear basal apparatus. The latter, however, ultimately becomes cellular. The embryo development is of the Solanad type. The mature seed has only two layers of thin-walled endosperm cells and a wavy testa representing the persistent epidermis of the integument. The rest of the integument is consumed by the endosperm. The uneven surface of the testa results from unequal development of the integumentary parenchyma and not due to either unequal radial stretching of the epidermis or irregular growth of the endosperm as in *Andrographis serpyllifolia*.

Introduction

A brief illustrated account of the seed structure in the family Acanthaceae has been given by Netolitzky (6), who has regarded the epidermal ornamentation of the testa as the criterion for classification. Recently, Maheshwari and Negi (2), Phatak and Ambegaokar (7), Mohan Ram and Sehgal (5), Johri and Singh (1), and Mohan Ram (4) have also dealt with the postfertilization changes in the ovules of some Acanthaceae. Mauritzon's (3) observations on endosperm and embryo development in *Adhatoda vasica*, being rather sketchy, have prompted a reinvestigation.

Material and Methods

The material for investigation was obtained from plants growing on the Old Delhi ridge near the University campus, Delhi, India, from December to March, 1954–1957. Ovules and seeds at various stages following fertilization were dissected out of the fruits and fixed in formalin–acetic–alcohol. After the familiar procedures of dehydration and embedding, the material was sectioned at thicknesses varying from 8 to 18 microns. Heidenhain's haematoxylin and erythrosin were used for staining the sections. Examination of a very large quantity of sectioned material was necessary to study the earlier stages of endosperm formation because of a high degree of sterility.

Endosperms were dissected out under a stereoscopic binocular microscope and mounted in a mixture of acetocarmine and glycerine jelly. The dissection technique is very useful in understanding the morphology of the endosperm, for sectioning may often result in rupturing of the thin-walled free-nuclear portions of the endosperm and cause difficulties in reconstruction.

[1]Manuscript received July 21, 1960.
Contribution from the Department of Botany, University of Delhi, Delhi 8, India.

Observations

Endosperm

The mature embryo sac is crescent-shaped with its chalazal end swollen or pointed (Figs. 1–3). The synergids and the antipodal cells degenerate before gametic union and the female gametophyte is gorged with granules of starch (Fig. 2). The polar nuclei coalesce prior to fertilization at the chalazal region. The division of the primary endosperm nucleus is accompanied by a wall which partitions the embryo sac into a small chalazal and a larger upper chamber (Fig. 3). The latter by a transverse division sets aside an elongated micropylar and a small central endosperm chamber (Fig. 4). Thus an early stage of endosperm consisting of three primary cells is formed. Of these, the micropylar and the chalazal function as haustoria, a feature typical of the acanthaceous endosperm (Mauritzon 1934). The bulk of the endosperm is, however, formed by the products of the central endosperm chamber.

The chalazal haustorium enlarges and its nucleus divides into two (Figs. 5–8). One can attribute a nutritive role to the chalazal haustorium because of its intimate contact with the vascular supply of the ovule and its prominent, densely staining nucleoli. This haustorium persists till the initiation of the cotyledons, after which it degenerates.

The micropylar haustorium is usually binucleate and, by its aggressive activity, digests the surrounding tissue and extends in the direction of the funiculus (Figs. 7, 9, 10). The nuclei in this haustorium become hypertrophied, and densely stained chromatin bodies appear in them (Figs. 9, 10). The nucleoli enlarge and assume various shapes (Figs. 4, 6, 10). Figure 10 shows an unusual haustorium with as many as eight unequal nuclei. Of these, the four larger ones appear to have been formed by a division of each of the two original nuclei in the haustorium. The four smaller nuclei, on the other hand, probably do not belong to the haustorium.

The nucleus in the central endosperm chamber divides and the daughter nuclei undergo repeated synchronous divisions (Figs. 5–8). Meanwhile this chamber grows into a long and tapering structure (Figs. 7, 8, 11, 12). The nuclei distribute themselves throughout although, around the proembryo, they are more numerous. As many as 128 nuclei are produced in the central endosperm chamber before walls are laid down among them. Wall formation first commences in the neighborhood of the two haustoria and

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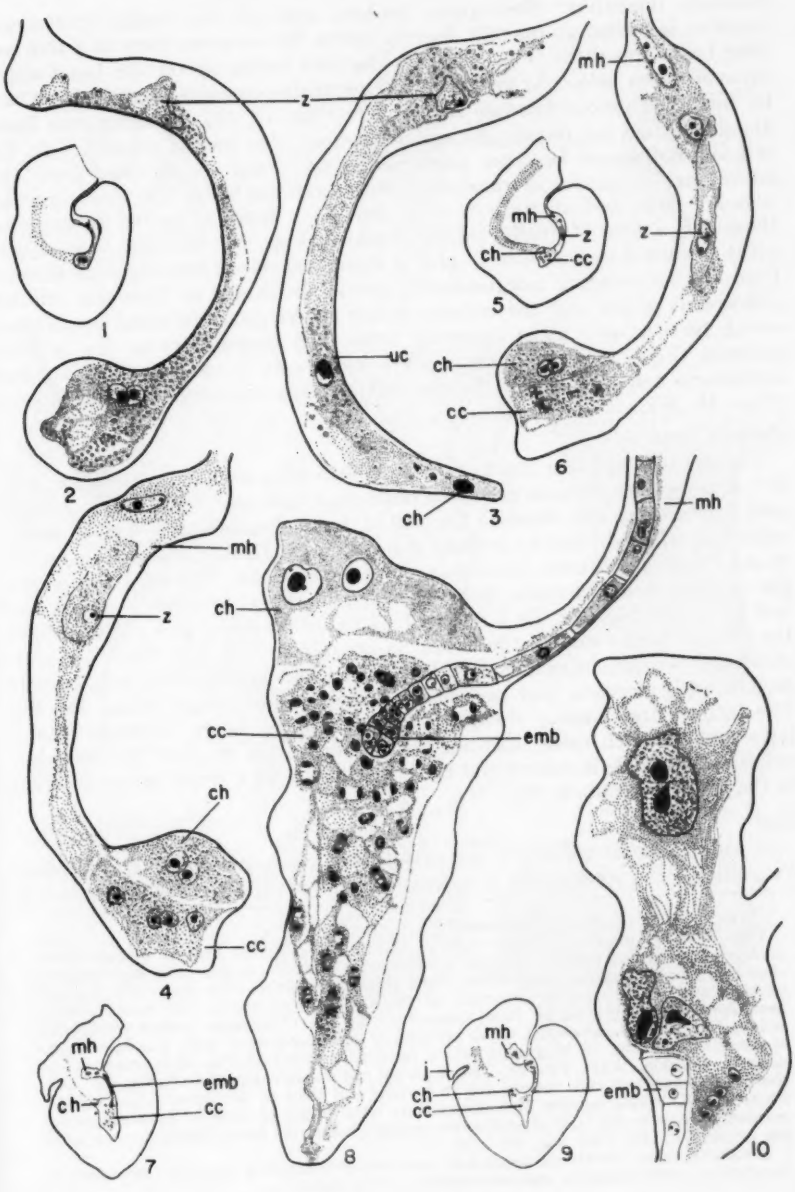
FIGS. 1–10. Endosperm.

FIG. 1. L. S. ovule with mature embryo sac. ×25. FIG. 2. Embryo sac enlarged from previous figure. Synergids and antipodal cells have degenerated. Starch is in abundance. ×275. FIG. 3. Two-celled endosperm. ×275. FIG. 4. Three-celled endosperm showing a 4-nucleate central endosperm chamber, a uninucleate micropylar haustorium, and a 2-nucleate chalazal haustorium. ×275. FIG. 5. Outline diagram for Fig. 6. ×25. FIG. 6. Endosperm enlarged from previous figure. All four nuclei in central chamber in division. The zygote is tubular and shows two nuclei. ×275. FIG. 7. Ovule at octant embryo stage. ×20. FIG. 8. Part of endosperm enlarged from Fig. 7. The central chamber has undergone enormous downward growth. The nuclei can be seen in various stages of division. Embedded in the endosperm proper is an octant embryo with a long suspensor. ×275. FIG. 9. L. S. young seed. ×20. FIG. 10. Micropylar haustorium enlarged from Fig. 9. Mark four hypertrophied and irregular nuclei and four smaller nuclei. Part of the embryo sac can also be seen. ×275.

[ABBREVIATIONS: *cc*=central endosperm chamber; *ch*=chalazal haustorium; *emb*=embryo; *j*=jaculator; *mh*=micropylar haustorium; *uc*=upper chamber; *z*=zygote.]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descends thereafter. The upper, broader part of the central endosperm chamber is walled up quickly, leaving below the narrower part as a free nuclear basal apparatus (Figs. 11, 12). The cells bordering on the basal apparatus are open below. At first the basal apparatus contains a few nuclei, which by further divisions increase in number (Figs. 13, 14). However, the basal apparatus does not remain nuclear throughout. The part in contact with the cellular endosperm becomes partitioned first (Figs. 15, 16) and soon the entire basal apparatus is transformed into a cellular body. The newly formed cells are large and uninucleate (Fig. 16). As a result of active divisions in these cells a mass of smaller cells is obtained (Figs. 17, 18). The basal apparatus as such does not seem to play a significant role in drawing nourishment from the surrounding integumentary tissue. In course of time the cellular endosperm proper and the embryo within it overgrow the basal apparatus, which persists as a short appendix below and degenerates as the embryo matures (Figs. 17–20). In the mature seed, only a two- to three-layered endosperm can be noticed, the rest having been consumed by the embryo (Figs. 38, 39).

Embryo

The zygote elongates enormously before dividing into cells *ca* and *cb* (Fig. 21). A transverse division ensues in both these cells and a proembryo of four cells *l*, *l'* and *ci*, *m* is formed. The cell *ci* divides immediately into *n'* and *n* while the cell *m* divides to produce *d* and *f* (Figs. 22, 23). The derivatives of *ci* and *f* contribute to the formation of a long suspensor. The cell *l* first undergoes a longitudinal division (Figs. 24, 25) and this is followed by a vertical wall in *l'* also (Fig. 26). By a further longitudinal division at a right angle to the first, in both *l* and *l'*, the octant is organized (Fig. 27). Eventual anticlinal and periclinal divisions differentiate the three embryonal layers, namely dermatogen, plerome, and periblem, in the globular stage (Figs. 28, 29). The latter, after passing through the heart-shaped stage, develops into a large embryo with thick, leaf-like cotyledons (Figs. 30, 31). In the adult embryo the radicle is curved and the cotyledons bear a small hump adjacent to the tip of the radicle (Fig. 31).

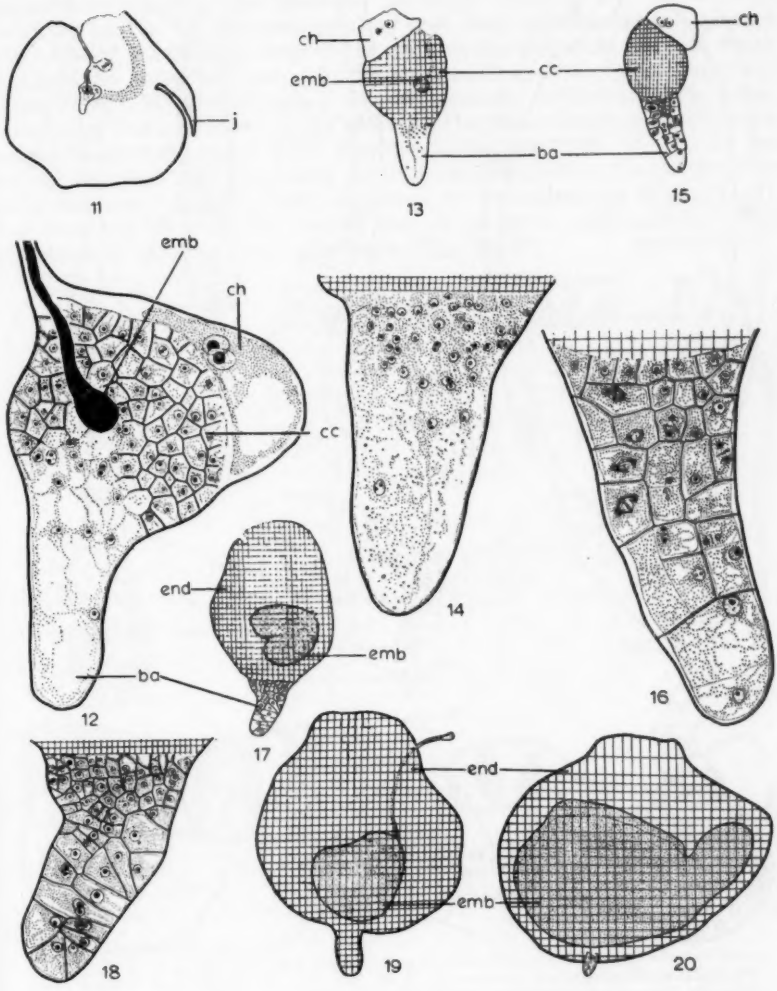
Seed

In each locule of the ovary are present two anatropous unitegmic ovules. At anthesis, the integument is nearly 40 layered; the cells are thin-walled

Figs. 11–20. Endosperm (*Concluded*).

FIG. 11. L. S. ovule at globular embryo stage. ×30. FIG. 12. Portion of endosperm enlarged from Fig. 11 to show an upper cellular and a lower free nuclear central endosperm chamber, a broad chalazal haustorium, and a globular embryo. A few open cells abutting on the nuclear part may be observed. ×148. FIG. 13. Endosperm with multinucleate basal apparatus. ×33. FIG. 14. Basal apparatus from Fig. 13 enlarged to show the details. ×148. FIGS. 15, 17. Dissected whole mounts of endosperm each with a cellular basal apparatus. ×33. FIG. 16. Magnified view of basal apparatus from Fig. 15 showing several nuclei in division. ×148. FIG. 18. Cellular basal apparatus enlarged from Fig. 17. Note the larger number of cells toward the embryonal end than at the opposite end. ×148. FIGS. 19, 20. Whole mounts of endosperm at later stages of embryo development. A marked increase in the bulk of endosperm proper renders the basal apparatus as a small tag below. FIG. 19, ×33. Fig. 20, ×17.

[ABBREVIATIONS: *ba* = basal apparatus; *cc* = central endosperm chamber; *ch* = chalazal haustorium; *emb* = embryo; *end* = endosperm; *j* = jaculator.]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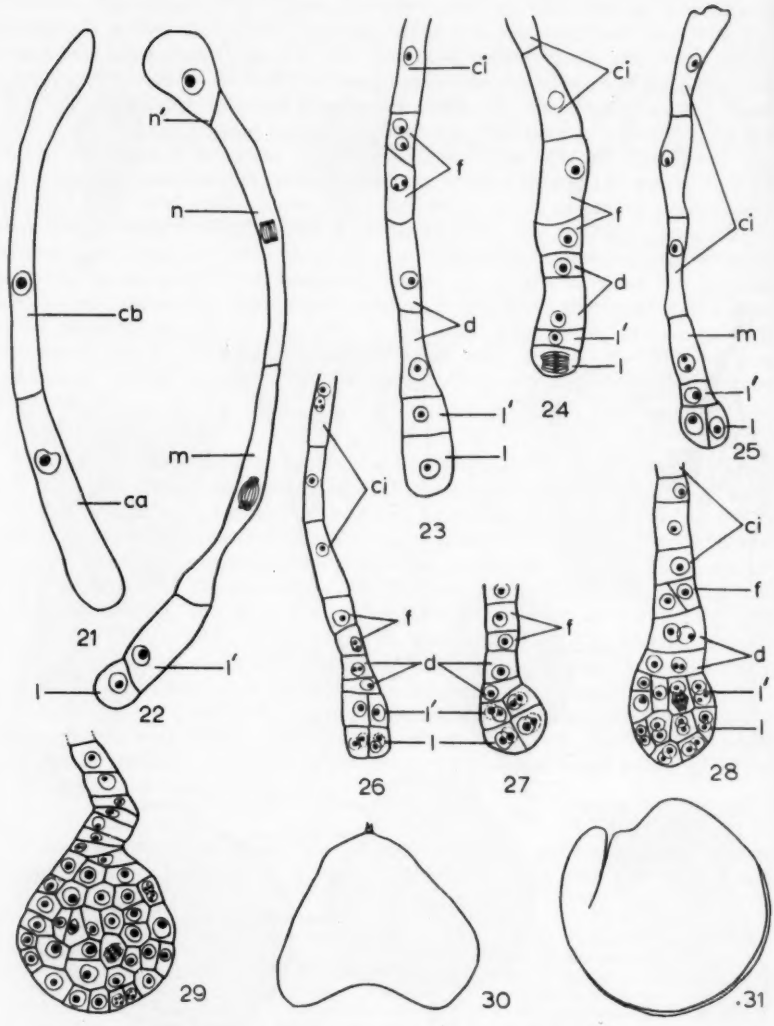
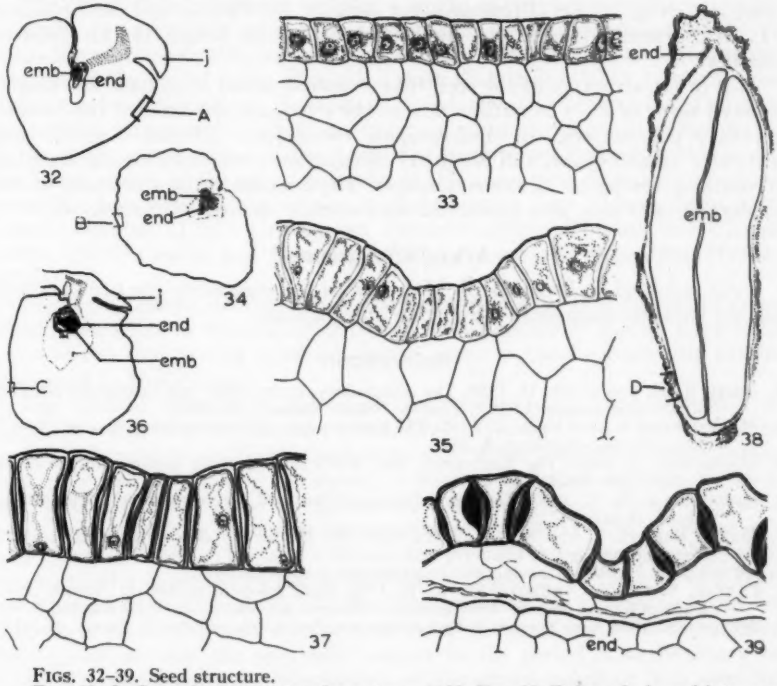


FIGS. 21–31. Embryo.

FIG. 21. Two-celled proembryo. ×367. FIG. 22. Five-celled proembryo. ×367. FIG. 23. Multicelled and filamentous proembryo. ×367. FIG. 24. Terminal cell, *l*, in division. ×367. FIG. 25. Vertical wall dividing *l* into two juxtaposed cells. ×367. FIGS. 26–29. Stages in the formation of a globular embryo. ×367. FIG. 30. Heart-shaped embryo. ×158. FIG. 31. Mature curved embryo. ×15. (Details in the text.)

and isodiametric. The epidermal cells are usually smaller than the rest of the integumentary cells. A curved outgrowth—the jaculator—develops from the funiculus on the side away from the micropyle (Figs. 32, 36). At the stage exhibited by Fig. 32, the integumentary cells show considerable enlargement (Fig. 33). At the globular stage of the embryo, the surface of the seed which was formerly entire becomes wavy due to unequal growth of the integument (Figs. 34–36). Marked changes in the epidermis are noticed after the differentiation of the cotyledons in the embryo. The cells enlarge and their outer tangential and the radial walls develop thickenings (Figs. 37–39). In the mature seed, only the epidermis of the integument persists as the rudimentary testa, the rest of it having been consumed by the endosperm (Figs. 38, 39). The testa has an undulated surface and its epidermal cells exhibit a thick deposition of lignin on their radial walls (Figs. 38, 39).



FIGS. 32–39. Seed structure.

FIG. 32. L. S. ovule at octant embryo stage. ×29. FIG. 33. Enlarged view of integument marked A in Fig. 32. × 367. FIG. 34. L. S. ovule at globular embryo stage. ×20. FIG. 35. Area marked B from Fig. 34 is magnified to show the radial elongation and greater vacuolation of the epidermal cells. ×240. FIG. 36. V. S. ovule at the torpedo stage of the embryo. ×20. FIG. 37. Magnification of the portion C in Fig. 36 to illustrate the thickenings of the radial walls of the epidermis. ×240. FIG. 38. T. S. mature seed. ×20. FIG. 39. Region denoted D in Fig. 38 in detail. The wavy epidermis has developed conspicuous thickenings; the rest of the integument has been used up. Two layers of endosperm are persistent. ×240.

[ABBREVIATIONS: *emb* = embryo; *end* = endosperm; *j* = jaculator.]

Discussion

The embryological events following fertilization in the ovules of *Adhatoda vasica* are somewhat typical of the family Acanthaceae. The rare occurrence of an 8-nucleate micropylar haustorium is reminiscent of the multinucleate micropylar haustorium in *Beloperone angustiflora* reported by Mauritzon (3). The large nuclei with very prominent nucleoli indicate that endomitoses probably occur in them. Steffen (8) has concluded on the basis of volume relationships that the nuclei of micropylar haustoria in *Pedicularis palustris* are about 384-ploid, while those of the chalazal haustorium are around 96-ploid. The tubular and multinucleate basal apparatus which ultimately becomes cellular is similar to that in *Justicia simplex*, studied by Mohan Ram and Sehgal (5).

The mode of embryo development resembles that described by Maheshwari and Negi (2) for *Dipteracanthus patulus*, by Phatak and Ambegaokar (7) for *Barleria prionitis*, and by Mohan Ram and Sehgal (5) for *Justicia simplex*.

It is in the structure of the seed that *Adhatoda vasica* is unique. The single-layered testa is wavy in outline due to the unequal expansion of the integumentary parenchyma. In *Andrographis serpyllifolia* (Mohan Ram 4) and *Elytraria acaulis* (Johri and Singh, 1) corrugation of the seed results from an irregular development of the endosperm. The unequal radial stretching of the epidermal cells may also render the seed surface uneven (Netolitzky 6).

Acknowledgments

I am indebted to Professor P. Maheshwari for suggesting the problem and for his valuable comments during the investigation.

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8. Steffen, K. 1956. Endomitosen in Endosperm von *Pedicularis palustris* L. Planta, **47**, 625.

SELF-FERTILITY IN THE SCLEROTINIACEAE[1]

J. Walton Groves and Mary E. Elliott

Abstract

Nine species representing five genera of the Sclerotiniaceae were found to be self-fertile. These are as follows: *Ciboria acericola* sp. nov., *C. acerina* Whetz. & Buchw., *C. amentacea* (Balb. ex Fr.) Fckl., *C. betulicola* sp. nov., *C. caucus* (Rebent. ex Fr.) Fckl., *Ciborinia erythronii* (Whetz.) Whetz., *Rutstroemia paludosa* (Cash & Davidson) comb. nov., *Septotinia podophyllina* Whetz., and *Verpatinia calthicola* Whetz. Two new species and one new combination are proposed and validating Latin diagnoses are provided for *Ciboria acerina*, the genus *Septotinia*, and *S. podophyllina*.

The family Sclerotiniaceae includes a group of inoperculate Discomycetes in which the apothecia arise from a stroma or stromatized tissue. Many of them are of considerable economic importance and they are also of interest because of the varied types of conidial state that they exhibit. It was in one of the species of this group, *Stromatinia gladioli* (Drayt.) Whetz., that Drayton (1932) first demonstrated the sexual function of the microconidia or spermatia in the Ascomycetes and as a direct result of the interest aroused by this work, the investigation of the sexual behavior of this group was extended to include other species. Some of the results of these studies have already been published, Drayton (1937), Groves and Drayton (1939), Drayton and Groves (1943, 1952), Groves and Loveland (1953), Groves and Bowerman (1955*a*, 1955*b*).

The main interest at first was in the mechanisms for bringing about cross fertilization, but in the course of the study a number of species have been encountered that proved to be self-fertile, and it is these species that will be discussed in this paper.

The method followed in the experimental series has been described in detail in earlier papers, Drayton (1934, 1937), Groves (1956). Briefly it is to grow the fungus on sterilized wheat in the dark under controlled temperatures, usually 5° and 14° C, until the stroma is mature. If the stroma is in the form of discrete sclerotia, these are picked off individually and placed on sterile moist sand, or if this cannot be done, pieces of the wheat culture are cut out and placed on sand. The sand cultures are given a rest period at 0° C, then spermatized and placed in the greenhouse exposed to north light at a temperature of approximately 10° C. The time required for a series is about six months or more and best results have been obtained when the series have been timed so that the apothecia mature in the period from February to April. To obtain accurate information on the sexual behavior it is essential that each isolate be kept in a separate dish throughout the entire experiment.

Self-fertility has already been demonstrated in a few species of this family.

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Contribution No. 111 from the Plant Research Institute, Research Branch, Department of Agriculture, Ottawa, Canada.

It has been reported in *Botryotinia ricini* (Godfrey) Whetzel by Godfrey (1923), in *Sclerotinia sclerotiorum* (Lib.) DeBary by Henson (1935), Keay (1939), and Coe (1944), in *S. minor* Jagger by Keay (1939), in *S. sativa* Drayton & Groves by Drayton and Groves (1943), in *S. borealis* Bub. & Vleug. by Groves and Bowerman (1955*b*), and in at least some isolates of *Ciborinia pseudobifrons* Whetz. by Groves and Bowerman (1955*a*). Whetzel (1943) in his monograph of *Lambertella* also reported self-fertility in *L. cornimaris* v. Höhn., *L. hicoriae* Whetz., *L. jasmini* Seaver & Whetz., *L. viburni* Whetz., and *L. cephalanthi* Whetz. The present paper adds nine species distributed through five genera to the known self-fertile species of the Sclerotiniaceae.

Ciboria acericola sp. nov. (Fig. 2, 7, 8, 16, 22*g*)

Apothecia 0.5–4 mm diam., in primis crateriformia, deinde applanata, tenua, fragilia; hymenium "Light Pinkish Cinnamon", "Cinnamon Buff", "Clay Color" [Ridgway Color Chart]; excipulum concolor a glabro minute pubescens; stipes 1–17 mm long., ad apicem ± 0.5 mm diam., gradatim attenuatus ad basas, concolor ad summas, pinguior ad basas, interdum paullum tomentosus ad basas; asci cylindrici, ad basas attenuati, (60) 70–80 (95) × (5.5) 6.5–8.0 (9.0) μ, octospori; ascospori hyalini, ab ellipsoideis paullum asymetrici, uniloculares, laeves, uniseriati, (6.5) 7.0–10.0 (11.5) × (2.5) 3.5–4.5 μ; paraphyses hyalinae, filiformes, septatae, simplices, vel ad basas ramosae, 1.5–2.5 μ diam., paullum ad summas incrassatae.

Habitat in foliis demissis *Aceris*, *Ulmi*, *Fraxini* (?), et filicis.

Ciboria acericola n. sp.

Apothecia 0.5–4 mm diam., at first cup-shaped, becoming flat expanded at maturity, thin, fragile, Light Pinkish Cinnamon to Cinnamon Buff or Clay Color (R.), outside of apothecium glabrous to minutely pubescent, concolorous with disc; stipe 1–17 mm long, about 0.5 mm in diameter at the apex, tapering gradually downwards, concolorous with disc above, becoming darker to blackish below, sometimes tomentose toward base; tissue of hypothecium prosenchymatous composed of ascending interwoven nearly hyaline hyphae about 2.5–3.5 μ diam., closely interwoven or with intercellular spaces, at the outside forming a poorly defined pseudoparenchymatous excipulum composed of one or two rows of thin-walled, irregular cells, sometimes grown out to form minute hairs, subhymenium a narrow, compact, yellowish zone of closely interwoven, slender hyphae; asci cylindric tapering below to a short stalk, eight-spored (60) 70–80 (95) × (5.5) 6.5–8.0 (9.0) μ; ascospores hyaline, ellipsoid to slightly flattened on one side, one-celled, smooth, uniseriate (6.5) 7.0–10 (11.5) × (2.5) 3.5–4.5 μ; paraphyses hyaline filiform, septate, simple or branched near the base, 1.5–2.5 μ in diameter, slightly enlarged toward the tip.

On fallen leaves of *Acer*, *Ulmus*, *Fraxinus* (?), and fern.

Type. DAOM 45956. On *Acer* leaves, Bell's Corners, near Ottawa, Ont., May 25, 1953. Coll. J. W. Groves, Sheila C. Hoare, and Constance A. Bowerman.

Specimens examined: Ontario: Bell's Corners, DAOM 45943, 45944, 45956, 65257, 65258, 65259, 65260; South March, DAOM 45979, 45980, 45981,

45983, 45984. Quebec: Gatineau Park, DAOM 65262. Apothecia produced in culture, DAOM 45964, 45969, 45977, 45986, 45987, 45992, 45993.

In the account of *Ciboria acerina* by Whetzel and Buchwald (1936), Whetzel mentions collecting a species with eight-spored asci on *Acer* along with *C. acerina*, and we believe *C. acericola* is probably this fungus. Our attention was first drawn to it in 1950 when one isolate in a culture series of *C. acerina* produced apothecia in abundance although none of the others did. Examination of these apothecia revealed that they all had eight-spored asci and on checking the original collection it was found to consist of a mixture of apothecia, some four-spored and some eight-spored.

The eight-spored form has since been collected several times and we agree with Whetzel's opinion that it is a species distinct from *C. acerina*. As well as being eight-spored, the asci and spores are slightly smaller than those of *C. acerina*. *C. acerina* is one of the early species to appear, our collections having been made in the period April 16–May 29, whereas *C. acericola* apparently comes later, our collections in the herbarium being made between May 20–June 1, although we have records of a few apothecia as early as May 10. Apothecia of *C. acericola* are produced much more abundantly in culture. Whetzel and Buchwald (1936) illustrated cultures of both species and although they are rather similar in appearance, with a little experience it is possible to distinguish them.

This species has been found most frequently on old leaves of *Acer* but it is evidently not restricted to this host. It has been found occasionally on *Ulmus* leaves, once on a leaf fragment thought to be *Fraxinus*, and once on a fern frond.

In culture the apothecia were slightly more robust, reaching 7 mm in diameter but mostly 3–6 mm, with the stipe up to 1 mm in diameter at the apex. The asci tended to be a little longer, 80–125 μ but about the same in width and the dimensions of the spores were about the same also with some slightly broader, up to 5.5 μ.

Four culture series were carried out with this species and apothecia were obtained quite readily in culture each time. Cultures were grown at both 5° and 14° C during the vegetative period and apothecia developed from both sets but in general the results were better with the isolates grown at 14°. In one series the period at 0° was omitted and the sand cultures stored for a corresponding period at 5° C. These cultures produced apothecia also. Apothecia developed in all cultures whether crossed, selfed, or left unspermatized and it is concluded that the fungus is self-fertile.

Ciboria acerina Whetzel & Buchwald, Mycologia, 28: 516. 1936 (Fig. 20, 22*e*)

Apothecium solitare vel plura, a late infundibuliformi parum profunde crateriforme, demum applanata, marginibus saepius recurvatis in maturitate; hymenium "Wood-Brown", "Avellaneous", ad "Vinaceous Buff" (Ridgway Color Chart); excipulum minute pubescens in juventute; stipes 1–10 mm long; a minute pubescente glabrum, paullum pinguior disco; asci breves et crassi, 75–107 × 7.5–8.8 μ, tetraspori; ascospori ellipsoidei, paullum asymetrici, hyalini, laeves, uniseriati 10–15 × 5–6 μ; paraphyses simplices, tenues, septatae, gradatim ad summas incrassati.

Habitat in inflorescentiis anniprioris *Aceris*, *Myricae*, *Salicis*, *Populi*, *Alni*, *Ostryaeque*.

Specimens examined: On *Acer*. Ontario: Ottawa District, DAOM 23242, 23243, 23244, 23337, 23338, 23339, 37536, 37544, 37546, 37551, 37573, 37582, 39425, 43498, 45937, 45938, 65261, 65263, 65265; Petawawa For. Exp. Stn., DAOM 26268; Holland R. Marsh, 64238, 64239. Quebec: Ottawa District, DAOM 4592, 5482, 5486, 5487, 12040, 23241, 37574, 57683. On *Alnus*. Ontario: Ottawa District, DAOM 60288, 63060, 63339. On *Populus*. Ontario: near Ottawa, DAOM 60289. New York: Ithaca, CUP 25237 (as *Ciborinia populicola* Whetz. inedit.) Apothecia developed in culture, DAOM 19518, 28501, 45972, 45996, 60853.

This species was well described and illustrated by Whetzel and Buchwald (1936) but unfortunately they failed to provide a Latin diagnosis and it is necessary to supply one now in order to validate the name. They reported that in two instances apothecia developed in culture on potato dextrose agar but they had no information regarding its sexuality.

Our measurements of the asci and spores are, in general, slightly smaller than stated by Whetzel and Buchwald (1936). Our measurements of the asci were (60) 70–80 (95) × (4.5) 6–8 μ, and of the spores (7) 8–12 (14) × (2.5) 3.5–5 (6) μ.

C. acerina is one of the common and more abundant members of the Sclerotiniaceae occurring in the Ottawa District. The fruiting period is about one month during the spring. Our earliest collection is on April 16 and the latest on May 26. We usually find it on *A. rubrum* occurring on the overwintered male inflorescence but we have also collected it on old petioles, leaves, bud scales, and in one instance on a small twig. Whetzel and Buchwald (1936) reported it as occurring on *Acer*, *Myrica*, *Salix*, and *Ostrya*, and we later received a culture from Professor H. M. Fitzpatrick from specimens on fallen needles of *Pinus strobus*, Menominee Indian Res., Shawans Co., Wisc. Coll. E. E. Honey, April 30, 1944. Det. Whetzel, No. S1615, CUP 33933, but we have not seen this specimen.

A specimen, No. CUP 25237, labelled *Ciborinia populicola* Whetz. in herb. was sent for examination by Dr. R. P. Korf. The original collection consisted of leaves of *Populus nigra* var. *italica* bearing sclerotium-like structures suggesting a *Ciborinia*. Isolations had been made from these sclerotia and apothecia produced from sclerotia developed in culture. The apothecia had four-spored asci in the size range of *C. acerina*. A number of two-spored and three-spored asci were observed and these contained abnormally large spores. The spores in the four-spored asci were similar to those of *C. acerina*. Whetzel's notes on the cultures could well apply to *C. acerina*. The apothecia themselves, although broader and with shorter stipes than usually found in *C. acerina* in nature, are similar in stature and general appearance to apothecia of *C. acerina* developed in our cultures. The stromata in the leaves are more sclerotium-like than is usually found in *C. acerina* in nature, but not more so than those formed in culture. We are of the opinion that this fungus is *C. acerina* and that *Populus* can also be added to the list of suscepts for this species. We have collected it once on *Populus* in the Ottawa District (DAOM 60289).

Five attempts have been made to produce apothecia in culture in this species and three of them have been successful. The failure in one series was probably due to the fact that the dishes in this series became heavily contaminated by another fungus, in the other the period of vegetative growth may have been too short and the stroma insufficiently developed.

The vegetative growth was carried out at 14° and 5°. Cultures grown at 5° for the entire period did not produce apothecia, but the best apothecium production was obtained in cultures grown for 1 month at 14°, then moved to 5° for a further 2 months. Apothecia were obtained in cultures in which the vegetative period was only 6 weeks but better results were obtained with longer periods. In one series the period at 0° was omitted and very good apothecium development was obtained.

Spermatium production was erratic and the factors controlling it are not clear. Sometimes spermatia were present but two of the series were not spermatized at all. Apothecia were obtained in both spermatized and unspermatized dishes, even when each isolate was kept in a separate dish.

Since this is a species with four-spored asci the question arises as to whether or not the apothecia may develop without fertilization. The fact that they were produced in cultures that were not spermatized and in series in which no spermatia were observed would support this idea, but it does not preclude the possibility that a few spermatia may have been produced and the cultures selfed.

Ciboria amentacea (Balbis ex Fr.) Fckl., Symb. Myc. p. 311, 1870 (Fig. 1, 11, 12, 22*d*)

Peziza amentacea Balbis, Mem. Acad. Turin II, p. 79. 1805.

Peziza amentacea Fries, Syst. Myc. 2: 126. 1822.

Rutstroemia amentacea Karst., Myc. Fenn. 1: 106. 1871.

Hymenoscypha amentacea Phill., Man. Brit. Disc. p. 120. 1887.

? *Peziza julacea* Pers., Myc. Eur. p. 311. 1822.

? *Helotium julaceum* Lambotte, Flor. Myc. Belg. p. 308. 1880.

Specimens examined: On *Alnus*. Ontario: Ottawa District, DAOM 2346, 43492, 43494, 45954, 56332, 56333, 56336, 60099, 60100, 60107, 60108, 63960; Holland R. Marsh, DAOM 64236, 64237, 70657, 70658, 70659, 70660, 70661, 70663; S. King City, DAOM 64235. Sweden: Flora Suecica 12109, ex UPS, DAOM 70662.

The culture series on *C. amentacea* were not very successful. In the five series attempted only a very few apothecia developed but they appeared in both spermatized and unspermatized dishes.

A very confusing situation exists with regard to the species of *Ciboria* occurring on male catkins of *Alnus*, *Populus*, and *Salix*. We realized about 1951 that there was a problem concerning the identity of the species on these hosts and since then we have collected and cultured these fungi intensively each spring. Over one hundred different collections have been made during this period. Single and mass ascospore cultures have been made from most of them. It is important to make mounts from the actual apothecium used to make ascospore cultures, but in most of the collections, mounts were made from each apothecium in the collection. Hence, several hundred apothecia have actually been examined.

As a result of this study we have come to the conclusion that there are two species of fungi occurring on these hosts and that they can be distinguished by the size of the ascospores. In one the ascospores rarely exceed 10 μ in length, mostly 8–10 μ, whereas in the other they usually exceed 10 μ and are mostly 11–15 μ long. There are other differences in width of spores, size of asci, and appearance of cultures but the spore length seems to be the most easily determined diagnostic character.

It is also evident from our observations that the small-spored species usually occurs on *Salix* and *Populus* and the large-spored species usually occurs on *Alnus*. It is sometimes very difficult to determine the identity of an old partly decayed, often fragmentary catkin, but we think we have at least six definite occurrences of the small-spored species on *Alnus*, and we have two very doubtful records of the large-spored one on *Populus*. We must admit the possibility at least of its occurrence on *Populus* and *Salix*.

It is noteworthy that the large-spored species can be found earlier in the spring than the small-spored one although there is considerable overlap in their fruiting periods. Our earliest collection date for the large-spored species is March 25 and the latest May 13, but it has rarely been collected after the middle of April, whereas the earliest date for the small-spored species is April 2 and the latest May 3 with most of the collections being made between April 10 and April 30. In our experience the large-spored species is one of the first members of the Sclerotiniaceae to appear in the spring. It is commonly found when snow is still on the ground and viable apothecia have been collected encased in ice. The only other species we have collected as early is *Ciboria alni* (Rostr.) Buchw. on female cones of *Alnus*.

These fungi have been called *Ciboria caucus* (Rebent. ex Fr.) Fckl. and *C. amentacea* (Balbis ex Fr.) Fckl. but the way in which these names have been used is confusing and it is difficult to decide their correct application.

C. caucus was originally described as *Peziza caucus* by Rebentisch, in 1804, and the name was validated by Fries (1822), who described it as occurring on catkins of *Populus alba*. Fuckel (1870) placed it in his genus *Ciboria* and it has been generally accepted as the type of the genus.

Ciboria amentacea was originally described by Balbis in 1805 as *Peziza amentacea* on alder catkins and Persoon (1822) also described *P. julacea* occurring on alder catkins. Fries (1822, p. 126) referred to these fungi in the following manner;

"Obs. *P. amentacea Balb. in Act. Ac. Tour. V. II*, t. 2. s. *P. julacea* Pers. *Myc. Eur.* p. 285 (ceres, brunnea, cupula hemisphaericopatula, stipite firmo, longitudine vario—*In amentis Alni glutinosae*) potissimum ad *P. tuberosam* spectat. Saltim similis forma loco citato a me visa."

Thus, although *P. amentacea* was not numbered or listed among the accepted species, it was published in the Systema with a diagnosis. This would seem to constitute valid publication of *Peziza amentacea* and we are accepting it as such. Fuckel (1870) also transferred *P. amentacea* to *Ciboria*.

The traditional view of these species is, therefore, that *C. caucus* occurs on *Populus* and *C. amentacea* on *Alnus* but we have no information about the spore size of the early collections. In our own collections of *C. amentacea* as we interpret it the asci measure (110) 125–160 (180) × 8–11 (12) μ and

the spores measure (8) 10–15 (17) × (3.5) 4.5–6 (8.0) μ, and in *C. caucus* the asci measure (95) 105–140 (160) × (5) 6.5–9 (10.5) μ and the spores measure (6.0) 7.5–10 (12) × (2.5) 3.5–4.5 (6) μ.

Nylander (1868) described *Peziza amentacea* on *Salix* and *Alnus* with spores 8–11 × 4.5–5 μ.

Fuckel (1870) described *Ciboria caucus* on *Populus* with spores 9 × 4 μ and *C. amentacea* on *Alnus* with spores 6 × 3 μ. This latter measurement does not agree with either of the species under consideration here and suggests that Fuckel had examined the much rarer *C. amenticola* (Karst.) Boud., also occurring on male catkins of *Alnus*.

Philips (1887) described these fungi in the genus *Hymenoscypha*, *H. caucus* on *Populus* with spores 10 × 6 μ and *H. amentacea* on *Salix* with spores 12 × 5.5 μ.

Rehm (1887–96) described *C. caucus* on *Populus* with spores 9–10 × 5–6 μ, and *C. amentacea* on *Alnus* and *Salix* with spores 9–12 × 4.5–5.5 μ.

Velenovsky (1934) described *C. amentacea* with spores 6–10 μ long and said *C. caucus* was the same.

Buchwald (1947) described *C. caucus* on *Populus* with spores 7.5–10.5 × 4.5–6 μ and *C. amentacea* on *Alnus* and *Salix* with spores also 7.5–10.5 × 4.5–6 μ.

Seaver (1951) described *C. caucus* on *Populus* and *Salix* with spores 9–10 × 5–6 μ and *C. amentacea* on *Alnus* and *Salix* with spores 9–13 × 4–5 μ.

Dennis (1956) described *C. caucus* on *Populus* with spores 7.5–10 × 4.5–6 μ and *C. amentacea* on *Alnus* and *Salix* with spores 7.5–10 × 4.5–6 μ.

Through the kindness of Dr. R. P. Korf it was possible to borrow the manuscript notes of the late Prof. H. H. Whetzel relating to these species. His notes contained descriptions of seven collections on *Alnus* catkins made in New York State with spore measurements of 10–22 × 5–11 μ. He also describes collections that he made in Europe, one in Holland, one in England, and the third presumably in England, and the spore measurements of these were 7.9–11.8 × 4.6–5.9 μ.

His notes indicate that he thought the species occurring on *Alnus* catkins in North America must have consistently larger spores than the European species and he had prepared a description of the American fungus as a new form of *Ciboria amentacea*. However, we know from specimens from Dr. J. A. Nannfeldt that the large-spored form does occur in Europe and we are of the opinion that Whetzel happened to collect *C. caucus* on *Alnus* in Europe.

There does not appear to be any type material of these species in existence at Uppsala or Leiden. Further search should be made in European herbaria to see if any material that could be designated as a lectotype is available. This choice should be made very carefully since the species are not necessarily restricted to either host, they are very similar in gross appearance, and there is a definite possibility of mixed collections.

In the meantime it seems best to follow the traditional view that *C. amentacea* is the common form occurring on *Alnus* and *C. caucus* the common form occurring on *Populus* and since we find the large-spored species most commonly on *Alnus* we are calling it *C. amentacea*, and the small-spored form, which occurs most commonly on *Populus*, is considered to be *C. caucus*.

This interpretation is based on the assumption that if a type of *C. amentacea* can be found it will prove to be the large-spored species and likewise that the type of *C. caucus* will prove to be the small-spored species. If types of these fungi are found and they should prove to be the same, the name *C. caucus* should have priority as it was published as a recognized species by Fries in the Systema whereas *C. amentacea* was only published there in a note. In that case *Peziza julacea* Pers. would have to be considered as a possible name for the species on *Alnus*. If types cannot be found it would seem desirable to designate types that would retain the name *Ciboria amentacea* for the large-spored species and *C. caucus* for the small-spored one.

The following is the key to the species of Sclerotiniaceae or very similar forms occurring on *Alnus*.

1. Apothecia sessile, opening by lobes..... *Pycnopeziza sympodiale* White & Whetzel
1. Apothecia stipitate, not lobed..... 2

2. Apothecia bright orange, drying red, on leaves..... *Ciboriella rufescens* (Kanouse) Seaver
2. Apothecia not red or orange..... 3

3. Apothecia yellowish green, on male catkins, asci 55–60 × 6 μ, spores 6–7 × 3 μ..... *Ciboria amenticola* (Karst.) Boud.
3. Apothecia yellowish brown, ochraceous, buff, or pallid, not green..... 4

4. Asci less than 75 μ long, on female cones..... *Ciboria uliginosa* (Fr.) Rehm
4. Asci mostly more than 100 μ long..... 5

5. Asci mostly more than 200 μ long, spores more than 20 μ long.... *Ciboria alnetorum* Vel.
5. Asci less than 200 μ long, spores less than 20 μ long..... 6

6. Spores becoming septate, on twigs or branches.. *Rutstroemia firma* (Pers. ex Fr.) Karst.
6. Spores one celled, not on twigs or branches..... 7

7. Spores 15–19 μ long, on female cones..... *Ciboria macrospora* Vel.
7. Spores mostly less than 15 μ long..... 8

8. Spores mostly less than 10 μ long, on male catkins. *Ciboria caucus* (Rebent. ex Fr.) Fckl.
8. Spores mostly 10–15 μ long..... 9

9. On seeds..... *Ciboria alni* (O. Rostr.) Buchw.
9. On leaves..... *Rutstroemia conformata* (Karst.) Nannf.
9. On male catkins..... *Ciboria amentacea* (Balb. ex Fr.) Fckl.

Ciboria betulicola sp. nov. (Fig. 6, 14, 15, 22*f*)

Apothecia 2–8–(10) mm diam., crateriformia, in primis profunda et margine incurvato, deinde expansa et plana, demum recurvata, tenua, fragilia, pallide griseo-brunnea cum margine paullum obscuriore; excipulum concolor glabrum; stipes longus, gracilis, tenuis, ad basas gradatim attenuatus, concolor ad summas, brunneus e nigro ad basas, 3–25 (35) mm long. et ± 0.5 mm diam. ad summas; asci cylindrici, ad basas attenuati octospori, (85) 95–125 (160) × (5) 6–9 (11) μ; ascospori ellipsoidei, hyalini, uniloculares, (5) 6–9 (11) × (2.5) 3.5–4.5 (6.5) μ; paraphyses hyalinae, filiformes, septatae, interdum ad basas ramosae, 1.5–2.0 μ diam., ad summas subclavatae.

Habitat in amentis masculis *Betularum* spp. et *Ostryae virginianae* (Mill.) Koch.

Ciboria betulicola sp. nov.

Apothecia 2–8 (10) mm in diameter, at first deep bowl-shaped with margin incurved, becoming shallow bowl-shaped, then flat expanded, and finally

recurved, thin, fragile, somewhat translucent, disc pale grayish brown, "Vinaceous Buff" to "Avellaneous" to "Wood Brown" (Ridgway), the margin slightly darker, excipulum concolorous, glabrous; stipe long, slender, tenuous, tapering gradually downwards, concolorous with the disc above, becoming darker to dark brown toward the base, 3–25 (35) mm long and about 0.5 mm in diameter at the apex; tissue of the hypothecium composed of three zones, an outer pseudoparenchymatous excipular layer two or three cells deep composed of irregular, thin-walled cells, 10–30 μ in diameter, a prosenchymatous medullary zone of rather loosely interwoven, hyaline hyphae 4–8 μ in diameter, and a very narrow subhymenium of closely interwoven, slender, hyphae with darker walls; tissue of the stipe prosenchymatous composed of ascending more or less vertically parallel hyphae forming a compact peripheral layer with a central medulla of slightly more loosely arranged and somewhat interwoven hyphae; asci cylindric, narrowed below to a short stalk, eight-spored (85) 95–125 (160) × (5) 6–9 (11) μ; ascospores ellipsoid, hyaline, one-celled, mostly uniseriate, sometimes partly biseriate, (5) 6–9 (11) × (2.5) 3.5–4.5 (6.5) μ; paraphyses hyaline, filiform, septate, sometimes branched near the base, about 1.5–2.0 μ in diameter, the tips slightly enlarged and clavate.

On male catkins of *Betula* spp. and *Ostrya virginiana* (Mill.) Koch. Type. DAOM 4455. On male catkins of *Betula lutea*, Labrador Lake near Tully, N.Y. Coll. H. H. Whetzel (S1221) and R. E. Fitzpatrick May 5, 1936. det. H. H. W. Cornell, Univ. Pl. Path. Herb. 25279.

Specimens examined: On *Betula*. Quebec: Burnet, DAOM 4590, CUP 26445; DAOM 12047, 37542, 37558, 57689; Kingsmere, DAOM 19829, 23245, 39426; Tenaga, DAOM 19835; Gatineau Park, DAOM 56312, 56322, 65256, 65266. Ontario: Merivale, 23248; Stittsville, 37566; Bell's Corners, 37567, 56311; Constance L., near Ottawa, 65264. Apothecia produced in culture DAOM 65255.

On *Ostrya*. Quebec: Burnet, DAOM 12039, 26244; Kingsmere, DAOM 12046.

This fungus was well known to Professor H. H. Whetzel and was carried in his notes under this name. However, he does not appear to have prepared a formal description and although his notes have been used in the preparation of the above description, it was felt that the responsibility of this description should be accepted by the present authors.

The fungus is common in the Ottawa district and can be collected regularly each season, usually on male catkins of *Betula* but sometimes also on *Ostrya*. The rather large, pale, fragile apothecia and small ascospores are characteristic. Our earliest collection date for this species is April 30, and the latest May 24.

On potato dextrose agar the cultures form a dense, white, cottony to felty, aerial mycelium with the submerged mycelium dark brown with a yellowish tint. The stromata develop as black, circular to irregular, anastomosing crusts.

The first attempt to obtain apothecia in this species was made in 1939. Apothecial fundaments were obtained in some isolates but the apothecia did not mature, and no further attempts were made with it until 1949. Since

then six different experimental series have been undertaken with this fungus. Spermatia were usually scarce although some isolates produced them in fair abundance. Apothecial fundaments were frequently produced but, as in the first series, they usually failed to mature. However some of them did mature including some in the unspermatized checks, hence it is concluded that the species is self-fertile.

The apothecia produced in culture were 2–4 mm in diameter, nearly flat, with the margin even or slightly crenate, darker brown than in nature, near Saccardo's Umber to Dresden Brown. The stipes were 6–10 mm long, shorter and slightly stouter than in nature, paler than the disc, and finely pubescent. The asci measured 110–180 × 8–10 μ and the ascospores 6–11 × 3.5–4.5 μ, which agreed very well with the dimensions observed in nature although the asci were, perhaps, slightly longer.

Ciboria caucus (Reb. ex Fr.) Fckl., Symb. Myc. p. 311. 1870 (Fig. 5, 13, 17, 22*h*)

Peziza caucus Rebentisch, Prodr. Fl. Neom. p. 386. 1804.

Peziza caucus Fries, Syst. Myc. 2: 126. 1822.

Phialea caucus Gill., Champ. Fr. p. 110. 1874.

Hymenoscypha caucus Phill., Man. Brit. Disc. p. 120. 1887.

Specimens examined: On *Populus*. Ontario: Ottawa District, DAOM 37540, 37549, 43496, 43497, 45939, 56317, 56318, 56331; Timagami Forest Reserve, DAOM 64233. Quebec: Ottawa District, DAOM 70872. On *Salix*: Ontario: Ottawa District, DAOM 37533, 43513, 45940, 45985, 56330, 60098, 60101, 60102, 60104, 70654; Ashbridge, Herts., England, ex K DAOM 39701. Sweden, ex UPS DAOM 70655, 70656. On *Alnus*: Ontario: Ottawa District, DAOM 43495, 56328, 60103, 60105, 60106, 63959. Quebec: Ottawa District, DAOM 70871.

For a discussion of this species see the notes on *C. amentacea*. Apothecia were obtained in six culture series of *C. caucus*, and they consistently appeared in unspermatized checks. In one series in which isolates of *C. amentacea* from *Alnus* were used along with isolates of *C. caucus* from *Populus* and *Salix*, apothecia were obtained in the isolates of *C. caucus* but not in those of *C. amentacea*. In another series isolates of *C. amentacea* from *Alnus*, and *C. caucus* from *Populus*, *Salix*, and *Alnus* were used. Here also apothecia were obtained in all isolates of *C. caucus*, including those from *Alnus*, but not in isolates of *C. amentacea*.

Ciborinia erythronii (Whetz.) Whetz., Mycologia, 37: 668. 1945 (Fig. 9, 18, 22*a*)

Sclerotinia erythronii Whetzel, Mycologia, 18: 232. 1926.

Specimens examined: On *Erythronium*. Ontario: Ottawa District, DAOM 37560, 45941, 65267. Quebec: Ottawa District, DAOM 4588, 5488, 12038, 19831, 23250, 23344, 37537, 37541. New York: Lloyd Preserve, McLean, DAOM 4458. Apothecia produced in culture, DAOM 28497, 28498, 28499.

C. erythronii occurs fairly commonly in the Ottawa District but the fruiting period appears to be relatively short. Our earliest record is April 30 and the latest May 16.

The species was well described and illustrated by Whetzel (1926). He studied it in culture but did not record any attempt to produce apothecia in culture.

In the culture series with this species it was found that apothecium production was definitely favored by keeping the cultures at 14° C during the period of vegetative growth on wheat. Apothecia were not produced in cultures grown at 5° C and the use of this temperature was abandoned after three trials.

Spermatia were produced in fair abundance on the wheat plates grown at 14° C, but usually some isolates produced them more abundantly than others.

Apothecia appeared in both selfed and crossed isolates and also in isolates which were not spermatized at all. The latter were probably selfed with spermatia developed in the culture. There is no doubt that the fungus is self-fertile.

Rutstroemia paludosa (Cash & Davidson) comb. nov. (Fig. 3, 21, 22*b*)

Sclerotinia paludosa Cash & Davidson, Mycologia, 25: 271. 1933.

Specimens examined: On *Carex*. Quebec: Gatineau Park, DAOM 4587, 43487, 56315, 56323, 56324, 60237, 64034, 64035, 65268, 65269. New York: Lloyd Preserve, McLean, DAOM 4463. Apothecia developed in culture, DAOM 57508.

This fungus occurs on old leaves of *Carex* and has been collected in the Ottawa District between May 9 and May 29. The apothecia are firm and robust with a prosenchymatous excipulum and spores which become septate. The asci measure (125) 145–160 (180) × (9) 10–12 (14) μ and the spores measure (9) 11–15 (17) × (3.5) 4.5–6 (6.5) μ. There is no conidial state and in culture a thin, narrow, black stroma is produced. It belongs in the genus *Rutstroemia* as defined by White (1941).

This species was first collected in the Ottawa District by F. L. Drayton in 1937. He sent some of the material to Prof. H. H. Whetzel who labelled it tentatively as an undescribed species of *Ciboria*. Prof. Whetzel stated that he had collected the same fungus in New York and he sent some specimens from the Lloyd Preserve, McLean, N.Y. These are now deposited in the Mycological Herbarium of the Plant Research Institute, Research Branch, Central Experimental Farm, Ottawa.

After further study of this fungus Whetzel stated in a series of letters to Dr. Drayton in 1942 that he had found the same fungus on *Acorus calamus* and *Eleocharis palustris*, that he now considered it to be a *Rutstroemia* rather than a *Ciboria*, and that he thought it might be the same fungus as *Sclerotinia homoeocarpa* Bennett and *Ciboria armeriae* v. Höhn. In these letters he made no mention of *Sclerotinia paludosa* Cash & Davidson (1933) described on leaves of *Carex*, and he probably did not consider this fungus because the original account indicated that the apothecia arose from sclerotia. When he examined the type material later he found that the sclerotia belonged to a *Typhula* and the apothecia actually arose from stromatized leaf tissue and he recognized it to be a *Rutstroemia* (Whetzel 1946). He thought that it was probably conspecific with *Sclerotinia homoeocarpa* Bennett.

We are in agreement that this fungus is *Sclerotinia paludosa* Cash & Davidson and that it properly belongs in the genus *Rutstroemia* as discussed by

White (1941). However, there is considerable doubt concerning its identity with *Ciboria armeriae* and *Sclerotinia homoeocarpa*.

Ciboria armeriae was described by von Höhnel (1918) as occurring on *Armeria vulgaris*. The size of the asci, 140 × 8 μ, and the ascospores, 13–14.5 × 5–6.5 μ, as published by von Höhnel, is close to that of *Rutstroemia paludosa*, but the apothecia were described as wine red, which is certainly not true of *R. paludosa*, and the host plant, *Armeria*, is not close to *Carex*. The basis for Whetzel's suggestion is apparently to be found in a letter to Drayton dated July 18, 1942, in which he stated that Dr. W. L. White had compared specimens of the *Carex* fungus with the type of *C. armeriae* at Harvard and thought that they were the same. However, in view of the difference in hosts and also in the color of the apothecia, which might not be evident in dried material, it would seem premature to place these names in synonymy. The fungus on *Armeria* should be collected and restudied in fresh condition and in culture before a final decision as to their identity is reached.

Sclerotinia homoeocarpa was described by Bennett (1937) as the organism causing dollarspot disease of grasses. He apparently did not find apothecia in nature but they were produced in his cultures. Two strains produced apothecia which differed considerably in the dimensions of the asci and spores, but one of them was close to *R. paludosa*. Again the basis for Whetzel's surmise regarding the identity of *S. homoeocarpa* and *R. paludosa* can be found in a letter to Drayton dated July 8, 1942. In this letter he stated that he had received some cultures of *S. homoeocarpa* from Bennett and was struck by their great similarity to those of the *Rutstroemia* on *Carex*. However, he admitted that he had not seen apothecia of *S. homoeocarpa*, and the evidence for its identity with *R. paludosa* is not very convincing. As well as the fact that *S. homoeocarpa* attacks Gramineae rather than Cyperaceae, there is the remarkable conidial state described by Bennett and which, so far at least, has not been observed in *Rutstroemia paludosa*.

It would seem better, therefore, to retain *Ciboria armeriae* and *Sclerotinia homoeocarpa* as autonomous species but they may well properly belong in the genus *Rutstroemia*.

One attempt was made to develop apothecia in culture from isolates of the 1937 collection but, although some apothecial fundaments appeared, they did not mature. This was thought to be possibly the result of failure of fertilization because no spermatia were found in the cultures and no spermatization was carried out.

The fungus was collected again in 1955 and 1956 and three experimental series have been carried out using isolates from these two collections. In the first series, we again failed to find spermatia and the dishes were divided into three groups. In one group, the isolates were painted with sterile soil extract and the brushes were not sterilized between isolates in the hope that spermatia might be transferred from one isolate to the other. All of the isolates in this group were painted in order using the same brush, and then done a second time in the reverse order using the same brush. In the second group, they were painted with sterile soil extract but the brushes were sterilized after each isolate was done. In the third group, the isolates were left untouched.

The results were unsatisfactory but apothecia matured in two dishes and it was indicated that the species was self-fertile.

In a second series, spermatia were found although they were very scarce. The cultures were grown at 5° and 14° C and eight single ascospore cultures were used as females and three as males. Apothecia matured in every dish except two, including the unspermatized dishes. Their failure to develop in two of the dishes may have been due to the presence of contaminants, but the species is undoubtedly self-fertile. This conclusion was confirmed in the third series.

A remarkable feature of this fungus was that the apothecia produced in culture were very much larger and more robust than those found in nature. They did not look like the same species at all but their identity was confirmed by single ascospore cultures. This is the only species of *Rutstroemia* in which we have obtained apothecia in culture. It would be interesting if other species of this genus exhibited the same tendency to produce larger and more robust apothecia in culture.

Septotinia Whetzel, Mycologia, 29: 134. 1937

Conidia hyalina, elongata, septata, in ramosis hyalinis conidiophoris natis, in sporodochio aggregata; sclerotia angulosa, elongata vel circularia, tenua, nigra; spermatia ovata, minutissima; apothecium haud profundum, cupulatum, stipitatum, ex sclerotiis natum; asci graciles, cylindrici; ascosporae hyalinae, ovoideae; paraphyses simplices vel ramosae, capitatae.

Septotinia podophyllina Whetz., Mycologia, 29: 135. 1937 (Fig. 10, 22*c*)

Conidia ad basas truncata, ad summas attenuata, 0–4 septata, recta vel curvata, 12.5–45.0 × 5.0–7.5 μ; conidiophorae in sporodochiis minutis, albis, mucilentis si humidis, cartilagineis et colore succineis si siccis, aggregatae; sclerotia 3–5 mm long., 1 mm lat.; spermatia minutia, hyalina, ovata, 2.4–3.6 × 1.5–2.1 μ, annulo brevi; apothecia 1–4 mm lat., pallide griseo-lutea, stipite ad basas attenuato, laevi, concolore; asci octospori, 116–148 × 7.5–10.0 μ; ascosporae continuae, 10–16 × 5–6 μ; paraphyses tenues, septatae, irregulariter capitatae.

In foliis *Podophylli peltati* crescit. Typus Plant Path. Herb. Cornell Univ. 25277.

Specimens examined: On *Podophyllum peltatum*. New York: Lloyd Preserve, McLean, Whetzel S1216 (DAOM 4468). Apothecia produced in culture. DAOM 12035, 48068, 57674.

This species was described and illustrated by Whetzel (1937) but unfortunately he failed to provide a Latin diagnosis for either the genus *Septotinia* or the type species *S. podophyllina*. It is, therefore, necessary to supply the Latin diagnoses above in order to legitimize the names since the rule requiring Latin diagnoses went into effect Jan. 1, 1935.

Whetzel wrote the name of the species as *Septotinia podophyllina* (Ell. & Ev.) Whetz., which is also contrary to Article 69 of the Code of Nomenclature. The basonym *Gloeosporium podophyllinum* Ell. & Ev. was based on the imperfect state only and Whetzel was describing a new species and not making a new combination. It should be written as *Septotinia podophyllina* Whetz.

S. podophyllina has not been collected in the Ottawa district since the host plant does not occur this far north. The material used in the culture series was collected at The Lloyd Preserve, McLean, N.Y. (Whetzel S1216) and at Labrador Lake, N.Y. (Whetzel S1219) by H. H. Whetzel and F. L. Drayton in May 1936. Single ascospore cultures were made from this material and later in the same year Whetzel collected leaves of *Podophyllum* in the same locality at Labrador Lake with lesions and fruiting bodies of the conidial state and isolations were also made from the conidia.

Both conidial and ascospore cultures were used in the experimental series and apothecia were obtained in culture from both.

This species produced apothecia in culture rather readily. In one series, they were obtained from sclerotia simply removed from plates of potato dextrose agar, placed on sand, and spermatized. After a rest period of 3 months at 0° C they were placed at 7° C for approximately one month, and then moved to the greenhouse where apothecia matured in about two weeks.

In another series grown on wheat plates, a comparison was made between the effects of transferring single sclerotia to sand and cutting out pieces of the wheat plate culture. Apothecia were larger and more numerous on the separate sclerotia and it was observed that they developed better around the edges of pieces that had been cut out of the wheat plates.

Whetzel (1937) observed that there appeared to be two different types of culture in this fungus and suggested that they might prove to be different sexual strains. However, this is not the case for apothecia were produced in selfed, single ascospore cultures that had been kept in separate dishes throughout the entire experiment, hence it must be regarded as self-fertile.

Verpatinia calthicola Whetz., Mycologia, 37: 692. 1945 (Fig. 4, 19, 22*i*)

Specimens examined: Quebec: Tenaga, DAOM 57680. Apothecia produced in culture, DAOM 34542, 45991.

The genus *Verpatinia* Whetz., of which this is the type species, was erected by Whetzel (1945) based on material collected on *Caltha* near Ithaca, N.Y. He made reference to material collected in the Ottawa District at Tenaga, Que., by Groves and Conners in 1937, but was uncertain if it were the same species because of doubt concerning the identity of the host. There was no *Caltha* in the vicinity where this collection was made and we were of the opinion that it was probably on leaves of *Populus* but this was never established. Cultures were obtained from ascospores of this collection and, in a preliminary experiment, apothecia were obtained in culture.

Unfortunately, the cultures were lost due to a sulphur dioxide leak in the refrigerator in which they were stored and no definite information was obtained as to its sexual behavior.

The fungus was not found again until May 1953 when Mrs. Bowerman and Miss Sheila C. Hoare collected it at South March, Ont., this time in an area where there was abundant *Caltha palustris* L. Cultures were again obtained from ascospores and a more extensive experimental series was undertaken in which each single ascospore culture was kept throughout in a separate dish. Five single ascospore cultures were used as females. The cultures were grown on wheat at 14° C from July 30 to October 21, 1953, and the sclerotia were

then removed and placed on sand at 0° C. They were spermatized on December 14 using four single ascospore cultures as males and leaving one set unspermatized. They were then placed at 5° C until January 12, 1954, when they were moved to the greenhouse. Apothecia were present on February 12 and they developed in every dish, including the unspermatized ones and those that were selfed. The fungus is clearly self-fertile.

In spite of the absence of *Caltha* from the Tenaga station where the 1937 material was collected, it would appear from the morphological similarity of the apothecia and the similarity of the cultures, that this collection was also *V. calthicola* but it would seem that it is not confined to *Caltha* but must occur on some other host as well. Shortly after the 1937 collection was made at Tenaga, the ecology of the area was changed by road construction and the fungus has not been found there again.

Acknowledgments

The authors wish to acknowledge their indebtedness to Mrs. C. A. Bowerman, formerly Assistant Mycologist, Division of Botany and Plant Pathology, Science Service, Ottawa, who made a very considerable contribution to this study, especially in the investigation of *Ciboria acericola*, *C. acerina*, and *C. betulicola*. Dr. F. L. Drayton has maintained continued interest in the work and carried out some of the earlier culture series. The late Professor H. H. Whetzel provided material of *Septotinia podophyllina*, and Professor R. P. Korf kindly loaned us Professor Whetzel's manuscript notes. The Latin diagnoses were prepared by Dr. B. Boivin. The photographs in Figs. 20 and 21 were taken by the Bio-Graphic Unit, Canada Department of Agriculture.

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EXPLANATION OF FIGURES

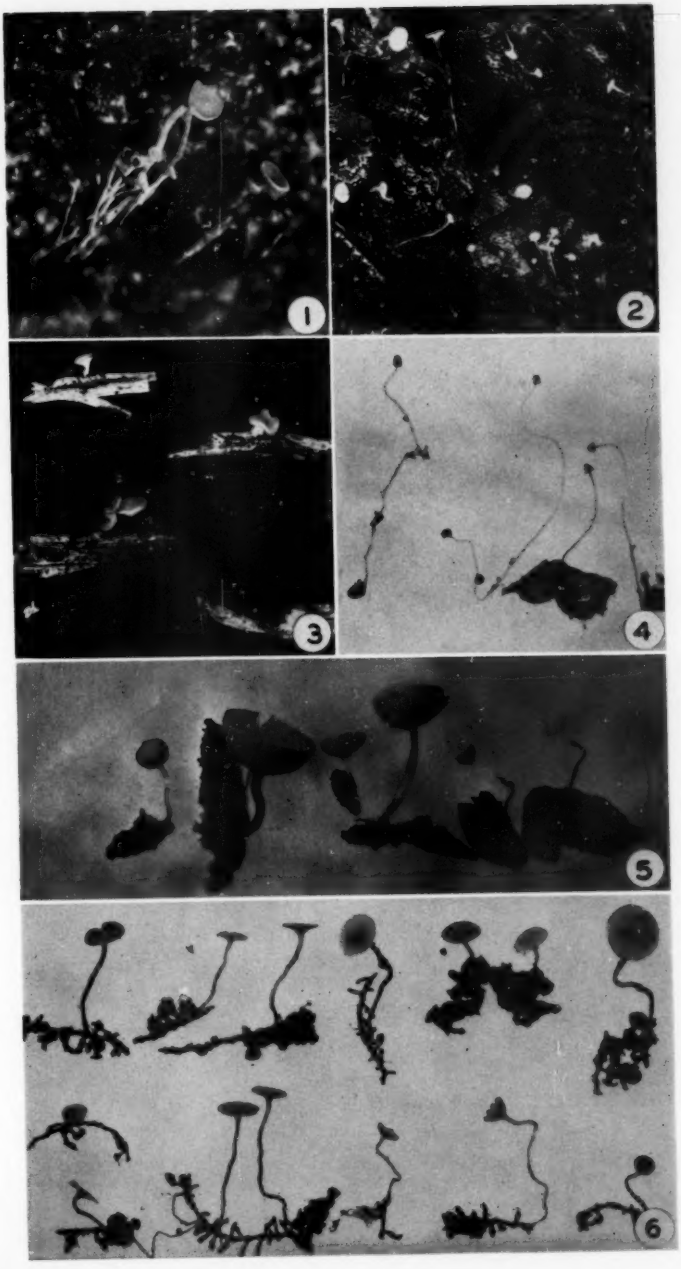
FIG. 1. *Ciboria amentacea*, × 2 approx.
FIG. 2. *Ciboria acericola* on leaf of *Acer*, × 2.
FIG. 3. *Rutstroemia paludosa*, × 1.
FIG. 4. *Verpatinia calthicola*, × 1.5.
FIG. 5. *Ciboria caucus*, × 2 approx.
FIG. 6. *Ciboria betulicola*, × 1.

FIG. 7. *Ciboria acericola*, apothecia from culture, × 1.
FIG. 8. *Ciboria acericola*, apothecia from culture, × 2.
FIG. 9. *Ciborinia erythronii*, apothecia from culture, × 1.
FIG. 10. *Septotinia podophyllina*, apothecia from culture, × 1.
FIG. 11. *Ciboria amentacea*, apothecia from culture, × 2 approx.
FIG. 12. *Ciboria amentacea*, apothecia from culture, × 2 approx.
FIG. 13. *Ciboria caucus*, apothecia from culture, × 2 approx.
FIG. 14. *Ciboria betulicola*, apothecia from culture, × 1.
FIG. 15. *Ciboria betulicola*, apothecia from culture, × 2 approx.

FIG. 16. *Ciboria acericola*, apothecia from culture, × 2.
FIG. 17. *Ciboria caucus*, apothecia from culture, × 2.
FIG. 18. *Ciborinia erythronii*, apothecia from culture, × 6.
FIG. 19. *Verpatinia calthicola*, apothecia from culture, × 2 approx.
FIG. 20. *Ciboria acerina*, apothecia from culture, × 2 approx.
FIG. 21. *Rutstroemia paludosa*, apothecia from culture, × 1.5 approx.

NOTE: Figs. 1–21 follow.

PLATE I



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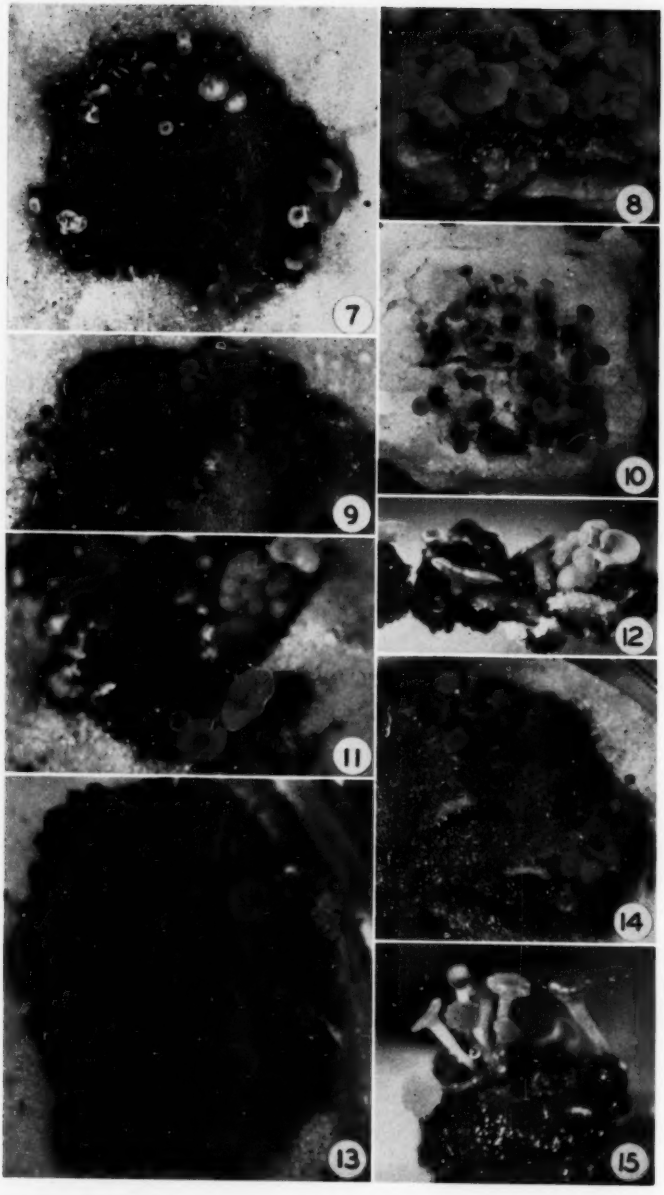

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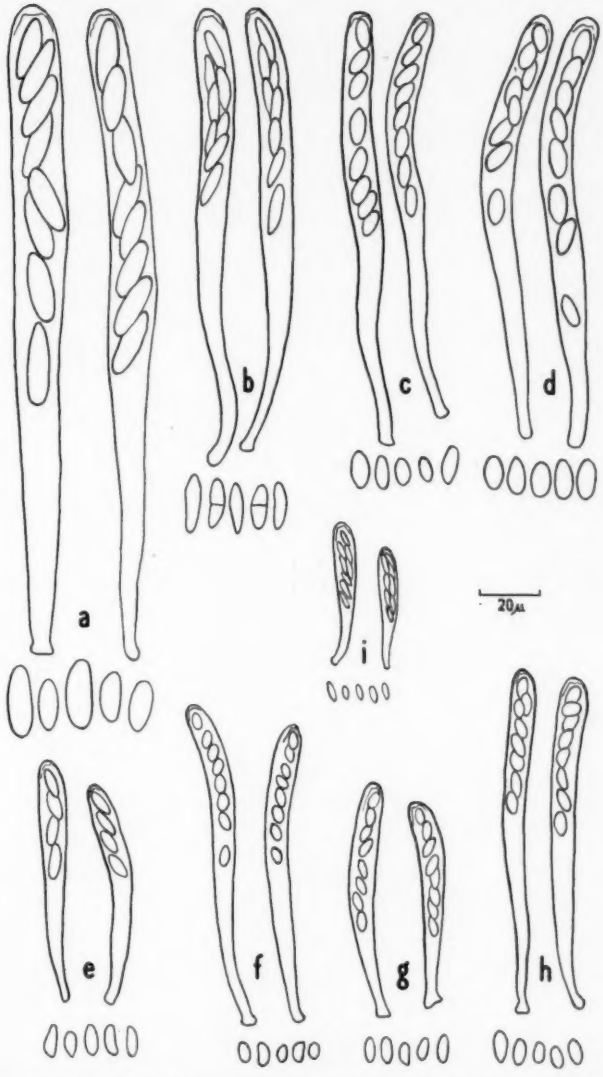

FIG. 22. Drawings of asci and ascospores. (*a*) *Ciborinia erythronii*; (*b*) *Rutstroemia paludosa*; (*c*) *Septotinia podophyllina*; (*d*) *Ciboria amentacea*; (*e*) *Ciboria acerina*; (*f*) *Ciboria betulicola*; (*g*) *Ciboria acericola*; (*h*) *Ciboria caucus*; (*i*) *Verpatinia calthicola*.

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